A

Thesis Report

On

Removal of Petroleum Hydrocarbons by using Microbial Mats

Submitted by

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Roll No. 210CH1045

In partial fulfillment of

Master of Technology (Chemical Engineering)

Under the Guidance of

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National Institute of Technology

Rourkela

2011

National Institute of Technology

Rourkela

CERTIFICATE

This is to certify that the report entitled, "Removal of Petroleum Hydrocarbons by using microbial mats" submitted by Miss Tanu Singh in partial fulfillments for their requirements for the award of Master of Technology Degree in Chemical Engineering at National Institute of Technology, Rourkela is prepared by her under my supervision and guidance.

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ACKNOWLEDGMENTS

The extensive endeavor, bliss euphoria that accompanies the successful completion of any task would not be complete without the expression of gratitude to the people who made it possible. So, I take the opportunity to acknowledge all those graceful people who always stood before me to provide the priceless guidance and encouragement to understand the existing technologies and helped me enthusiastically to head on towards the new technologies and research in the field of chemical engg.

I would like to express my sincere gratitude to Prof. (Dr) Susmita Mishra for her guidance and assistance in this summer report. The technical discussion with Prof. Mishra. was always been very insightful, and I will always be indebted to her for all the knowledge she shared with me. She always helped me in all the technical and non-technical issues during the production of this work. Her encouragement and efforts led this report to successful completion in a timely fashion.

I would also like to thank Prof. (Dr) B. Munshi for being a uniformly excellent advisor. He was always open minded, helpful and provided us with a strong broad idea and grateful acknowledgement is made to all the staff and faculty members of Chemical Engineering Department, National Institute of Technology, Rourkela for their consistent encouragement.

I would like also like to thank Prof. (Dr). R.K. Singh HOD of our department or his kind helps to make this report complete.

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ABSTRACT

Biodegradability of petroleum hydrocarbon was studied on the petroleum sludge from the industry (HPCL, Vishakhapatnam) by using microbial mat. Microbial mat is consisting of biological fiber like coconut fiber and jute fiber and it is immobilized by hydrocarbon degrading bacteria *Pseudomonas aeruginosa*. Adsorption study was performed on biological fiber by SEM, EDX and FTIR analysis. Biosurfactant screening was done on CTAB and Methylene Blue plate and it confirmed the production of Rhamnolipid. Optimum conditions like Temperature, pH and Nitrogen source for the degradation of the hydrocarbon was also studied by using Taguchi method. Total petroleum hydrocarbon of petroleum sludge degradation was studied for 4 weeks using microbial mat, and degradation of hydrocarbon was confirmed by GC-MS analysis and it shows predominant result under optimum growth conditions.

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CHAPTER - 1

INTRODUCTION

In the present scenario major environmental pollution of soil and water is due to hydrocarbon contamination resulting by the petrochemical industries activities. It can cause by accidental liberation of petroleum industries discharge in the environment or it can also cause by human activity. Hydrocarbon compounds are known for its carcinogenic and neurotoxic behavior. The overall annual intake of petroleum hydrocarbon around the world is very high and it is approximately about 1012 US gallons [1]. At higher concentration of the hydrocarbon molecules which are the main constituent of crude oil and petroleum products are highly toxic to living beings, including humans. Petroleum products also comprises trace amounts of sulfur and nitrogen compounds, which are hazardous and can react with the environment to produce secondary poisonous chemicals.

Petroleum and oil residues are complex mixtures of many compounds with a high proportion of hydrocarbons, which has different solubility and microbial resistances to biodegradation [2–4]. There are lots of method for treating petroleum contaminated sites such as Mechanical and Chemical methods, but these methods are generally have limited effectiveness and can be expensive. Bioremediation on the other hand is the promising technology for the treatment of these petroleum pollutant areas since it is cost-efficient and will result in to complete mineralization. Bioremediation functions basically on biodegradation, which may refer to absolute mineralization of hydrocarbon contaminants into carbon dioxide, water, inorganic compounds, and cell protein or conversion of complex organic pollutants to other simpler organic compounds by biodegradation agents like microorganisms [5-8].

From last decade, bioremediation of petroleum and oil-contaminated soils has draw much attention, and lots of research have been carried out with pure culture or mixed bacterial consortia isolated from oil-contaminated soils. Numerous microorganisms with the ability to degrade the hydrocarbons presented in oil have been reported. The researchers agreed to the opinion that biodegradation is an efficient and cost effective strategy. There are few single strains of microorganisms with the metabolic capacity to degrade all the components of crude oil. This may be due to the fact that a single strain could only degrade one species or certain types of compounds in crude oil, and many factors may affect the bioremediation of oil-contaminated soils, such as physical conditions, nutrition, the ratios of various structural hydrocarbons present, the bioavailability of the substrate and the diversity of the bacterial communities involved [9-10].

Among different compounds of petroleum oil, the most representative low-molecular-weight molecules, such as straight, branched, cyclic alkanes and aromatic hydrocarbons, were appear to be readily degraded by many microorganisms. On the other hand long-chain alkanes and polycyclic aromatic hydrocarbons (PAHs) were generally considered to be only slightly biodegradable due to their higher hydrophobicity [11].

Biodegradation of petroleum hydrocarbons is a very time taking and complex process that depends mainly on the nature and amount of the hydrocarbons present in the contaminants. Petroleum hydrocarbons can be classified into into four classes: the saturates, the aromatics, the asphaltenes: fatty acids, ketones and esters, etc., and the resins: pyridines, quinolines, carbazoles, sulfoxides, and amides [12]. Bioavailability is one of the important factors that limit biodegradation of oil pollutants in the environment by microorganisms. Petroleum hydrocarbon compounds bind to soil components, and they are difficult to be removed or degraded [13].

Different hydrocarbons have different susceptibility to microbial degradation and it takes different time span to degrade different hydrocarbons. The response of hydrocarbons to microbial degradation can be generally stratified as follows: linear alkanes > branched alkanes > small aromatics > cyclic alkanes [14, 15]. Some compounds, such as the high molecular weight polycyclic aromatic hydrocarbons (PAHs), may not be degraded at all [16].

Jones et al recognized the biodegraded petroleum-derived aromatic hydrocarbons in marine sediments. They investigated the immense biodegradation of alkyl aromatics in marine sediments which eventuate prior to detectable biodegradation of n-alkane profile of the crude oil and the microorganisms namely: *Arthrobacter, Burkholderia, Mycobacterium, Pseudomonas, Sphingomonas, and Rhodococcus* were observed to be involved for alkyl aromatic degradation. Adebusoye et al reported microbial degradation of petroleum hydrocarbons in a polluted tropical stream in Lagos, Nigeria. Nine bacterial strains namely: *Pseudomonas fluorescens, P. aeruginosa, Bacillus subtilis, Bacillus* sp., *Alcaligenes* sp., *Acinetobacter lwoffi, Flavobacterium sp., Micrococcus roseus, and Corynebacterium* sp. were isolated from the polluted stream which could degrade crude oil [17-21].

Biological fibers as Adsorbent of Petroleum Hydrocarbons

In order to remove the hazardous pollutants such as heavy metals, dye, hydrocarbons etc. adsorption has drawn great attention, especially processes which use low-cost adsorbing materials, such as biomass [22, 23]. The highly hydrophobic characteristics of biomass combined with its high porosity develop a capillary force towards the adsorption of oils. Vegetal tissues, with large surface area and big pores, tend to adsorb organic contaminants through physical and chemical mechanisms, in a similar way to charcoal [24–27]. Among the type of biomasses

employed as adsorbents are sea plants, cotton fibers, saw dust, corncob, coconut fibers, jute fibers and sugarcane bagasse. These materials can be used as a support for new adsorbents as well as being used "in-nature", representing thus a great reduction in costs [28–30]. Several researchers, while studying adsorption of oil by-products using different types of biomass, have observed the promising character of these materials as adsorbents [31, 32].

OBJECTIVE

The objective of this study is to develop a technology which is cheap, easy to handle and feasible and can clean up the oil spills and other aqueous environment.

Specific objectives

- Design a microbial mat by the use of biological waste fiber and hydrocarbon degrading bacteria.
- Examine the adsorption behavior by the waste biological fiber.
- Investigate the effect of parameters on the growth of petroleum degrading bacteria.
- Investigate the biosurfactant produce by the petroleum hydrocarbon degrading bacteria.
- To study the growth rate of bacteria during degradation study.
- Analyze the pattern of degradation of petroleum sludge by the petroleum degrading Bacteria.

CHAPTER - 2

LITERATURE REVIEW

Degradation of Petroleum Hydrocarbon has been studied by many researchers using different adsorbents like activated carbon, chemical method, charcoal, biological fiber, biological degradation etc. The study of adsorption and degradation by different microorganism and adsorbents is a burning challenge in present scenario on which a large number of researchers were working.

2.1 Biodegradation of Hydrocarbons by different Microorganism

Acevedo et al., 2010 evaluated the degradation of three and four-ring polycyclic aromatic hydrocarbons (PAHs) in Kirk medium by Anthracophyllum discolor, a white-rot fungus isolated from the forest of southern Chile, A. discolor was able to degrade PAHs in Kirk medium with the highest removal occurring in a PAH mixture, suggesting synergistic effects between PAHs or possible cometabolism.

Zhang et al., 2010 investigated a bacterial isolate, designated as DQ8, was capable of degrading diesel, crude oil, n-alkanes and polycyclic aromatic hydrocarbons (PAHs) in petroleum. Strain DQ8 was assigned to the genus Pseudomonas aeruginosa based on biochemical and genetic data. The metabolites identified from n-docosane as substrate suggested that P. aeruginosa DQ8 could oxidize n-alkanes via a terminal oxidation pathway.

Yousaf et al., 2011 investigated Enterobacter ludwigii all strains were capable of hydrocarbon degradation and efficiently colonized the rhizosphere and plant interior. Two strains, ISI10-3 and BRI10-9, appeared to degrade diesel fuel with highest rates and performed best in combination

with Italian ryegrass and alfalfa. All strains carry the CYP153 gene in all plant compartments, manifested an active role in degradation of diesel in association with plants.

Quek et al., 2004 observed that the ability of four different microorganisms to immobilize on PUF and to degrade various petroleum products was assessed by measuring the n-alkane fraction remaining in the petroleum products over time. A Rhodococcus sp. (designated as F92) had the highest number of immobilized viable cells (109 cells per cm^3 PUF) and a maximum attachment efficiency of 90% on PUF of a density of 14 kg/m^3.

2.2 Role of Biosurfactant in Biodegradation

Reddy et al., 2010 observed the bacterial strain PDM-3 has the ability to produce biosurfactant during phenanthrene degradation as detected by the surface tension measurements of the culture supernatant and the emulsification index (EI24). The biosurfactant was identified by its functional groups through FT-IR spectroscopy. Phenanthrene degradation and biosurfactant production are associated with each other and can be used in environmental biotechnology.

Hua et al., 2003 studied that the biosurfactant BS-UC was produced by Candida antarctica from n-undecane as the substrate. It was found that the addition of BS-UC influenced positively the emulsification and the biodegradation of a variety of n-alkanes substrates.

Ramana, et al., 2007 studied that on the biosurfactants produced by Pseudomonas aeruginosa CFTR-6 revealed that they consisted of glycolipids R-1 and R-2. The effect of variation of the media components such as carbon, nitrogen, phosphate and metal ions has been investigated. The

following values were found to be optimum for biosurfactant production: glucose, 20 g dm^{-3}; carbon to nitrogen ratio, 38; phosphate, 30 mmol dm^{-3}; $MgSO_4.7H_2O$, 100 mg dm^{-3}.

Calvo et al., 2004 studied the growth, biosurfactant activities and petroleum hydrocarbon compounds utilization of strain 28-11 isolated from a solid waste oil. The isolate was ascertained as Bacillus pumilus. It grew well in the presence of crude oil and naphthalene under aerobic conditions and consume these substances as carbon and energy source.

T. Priya and G. Usharani, 2009 investigated Biosurfactants are amphiphilic compounds produced by various bacteria and fungi which reduce surface and interfacial tension. Bacillus subtilis and Pseudomonas aeruginosa produces biosurfactants using vegetable oil, kerosene, petrol and diesel as source. The isolated biosurfactants were identified using TLC method.

Pinzon, et al., 2009 investigated rhamnolipid produced predominantly by Pseudomonas aeruginosa, are biosurfactants with important applications. For efficient culture screening according to rhamnolipid productivity, the method using agar plates containing methylene blue (MB) and cetyl trimethylammonium bromide (CTAB) was re-examined. Circles formed due to complexation between anionic rhamnolipids and cationic MB/CTAB is analyze by using a set-up, a fixed underneath light source and image analysis software.

2.3 Role of Natural Biofiber as an Adsorbent

Crisafully et al., 2007 investigated Removal of polycyclic aromatic hydrocarbons (PAHs) from petrochemical wastewater using various low-cost adsorbents of natural origin including sugar cane bagasse, green coconut shells, chitin, and chitosan. The adsorption isotherms of PAHs were in agreement with a Freundlich model, while the uptake capacity of PAHs followed the order:

green coconut shells > sugar cane bagasse > chitin > chitosan. The adsorption properties of green coconut shells were comparable to those of some conventional adsorbents such as Amberlite T.

Kumar et al., 2004 investigated that using phosphoric acid jute fiber is prepared as activated carbon. Feasibility of employing this jute fiber activated carbon (JFC) for the removal of Methylene blue (MB) from aqueous solution was investigated. Methylene blue adsorption on jute fiber activated carbon has investigated to dependent on contact time, MB concentration and pH.

Bhatnagar et al., 2010 observed that among several agricultural wastes studied as biosorbents for water treatment. Among all the bio-wastes coconut has been of great importance because its various parts (e.g. coir, shell, etc.) have been extensively studied as biosorbents for the eviction of diverse type of pollutants from water. Coconut-based bio-wastes have attracted wide attention as effective biosorbents due to low-cost and significant adsorption potential for the removal of various aquatic pollutants.

Hameed et al., 2010 studied the ability of coconut bunch waste (CBW) to remove basic dye (methylene blue) from aqueous solution by adsorption was studied. The experimental data were analyzed by the Langmuir, Freundlich and Temkin models of adsorption. The adsorption isotherm data were fitted well to Langmuir isotherm and the monolayer adsorption capacity was found to be 70.92mg/g at 30^0 C

Almeida de Sousa et al., 2009 observed lignocellulosic residues are interesting materials for the production of heavy metal adsorbents for aquatic systems. Whole fibers taken from coconut (Cocos nucifera) husks were functionalized with the thiophosphoryl (P=S) group by means of the direct reaction with $Cl_3P=S$, $(CH_3O)_2ClP=S$ or $(CH_3CH_2O)_2ClP=S$ in order to obtain an

adsorptive system for ‘soft’ metal ions, particularly Cd^{2+}. These functionalized fibers (FFs) were characterized by means of elemental analysis, infrared spectroscopy, thermal analysis and acid–base titration. Adsorption isotherms for Cd^{2+} fitted the Langmuir model, with binding capacities of 0.2–5 mmol g^{-1} of FF at 25^0 C.

Brandao et al., *2009* evaluated the adsorption ability of sugarcane bagasse to remove oil by-products from aqueous solution. Adsorption experiments were carried out in an agitated reactor at room temperature to obtain kinetic curves and adsorption isotherms of gasoline and n-heptane on sugarcane bagasse. The results showed the great potential of bagasse as an adsorbent, since it was able to adsorb up to 99% of gasoline and 90% of n-heptanes in solutions containing about 5% of these contaminants. In the adsorption kinetics of gasoline, the equilibrium was reached after just 5min. This result shows that the adsorption is very favorable. Langmuir, Freundlich, Temkin and D-R models did not describe well the adsorption behavior obtained for these systems.

Shukla et al., *2005* investigated the possibility of adsorbing Pb (II) from solution using coir, a cheap lignocellulosic fiber, was assessed in a fixed bed column. The coir fibers were also chemically modified by covalent loading of a reactive dye, C.I. Reactive Orange 13, and used as adsorbent. It is observed the column packed with dye treated fibers was performed longer duration than the one packed with unmodified coir fibers. In column packed with dye treated coir fibers Pb(II) adsorption was better than the unmodified coir packed column.

2.4 Factors effecting Growth of *Pseudomonas* species

Harjai et al., 2004 determined the effect of pH on production of extracellular virulence factors of *Pseudomonas aeruginosa* grown on catheter in biofilm. Alginate and proteinase production was higher at pH 8.

Abouseoud et al., 2007 studied *pseudomonas fluorescence* cultures conditions involving effect of difference in carbon and nitrogen sources and variation in C: N ratios were analyzed at constant temperature and pH, in order to increase the productivity of the process. The combination of olive oil and ammonium nitrate as carbon and nitrogen sources with a C: N ratio of 10 gives the best results.

Rahman et al., 2004 studied the physical factors affecting the production of an Pseudomonas aeruginosa strain K. Growth and protease production were detected from 37 to 45^0C with 37^0C being the optimum temperature for *P. aeruginosa*. Protease highest production observed at pH 7.0. but it can be detected over a broad pH range from 6.0 to 9.0.

2.5 Parameter optimization method

Kamaruddin et al., 2004 studied the optimal process parameters for an injection moulding machine using Taguchi method that was used to produce a consumer product i.e. plastic tray. In this study, both the optimal process parameters for injection moulding process and main process parameters that affect the bending performance of the tray can be found.

Velsco et al., 1994 describes a statistical criterion for estimation and selection of different testing methods for solid biofuels taking into consideration accuracy, precision, sensitivity,

reproducibility, repeatability, testing costs and testing time. The signal-to-noise ratio as given by Taguchi has been used in the similar manner to a traditional method (analysis of variance. ANOVA) suggested for this purpose.

Daneshvar et al., 2006, investigated the optimization of biological decolorization of synthetic dye solution containing Malachite Green. Effect of different parameters such as temperature, initial pH of the solution, type of algae, dye concentration and time of the reaction was investigated and optimized using Taguchi method. For the study of biodegradation of the dye total sixteen experiments were required. Based on the S/N ratio, the optimized conditions for dye removal were temperature 25^0 C, initial pH 10, dye concentration 5 ppm, algae type Chlorella and time 2.5 h.

Dasu et al., 2002 Taguchi's method was applied to evaluate the significant parameters for griseofulvin production by Penicillium griseofulvum MTCC 1898 in a batch bioreactor. Physical parameter, controlled pH, agitation and aeration had a significant influence on griseofulvin production when compared with chemical parameters and were considered as significant ones.

2.6 Mechanism of Petroleum Hydrocarbons Degradation [33]

Under aerobic condition, the most rapid and complete degradation of the majority of organic pollutants occurred. The first intracellular reaction is an attack on the organic pollutants and it is an oxidative process, this is an enzymatic reaction in which incorporation and activation of oxygen is done and is catalyzed by oxygenases and peroxidases enzymes. A peripheral degradation pathway is a step by step process and it converts organic pollutants gradually into intermediates of the central intermediary metabolism, for example, the tricarboxylic acid cycle.

Central precursor metabolites, for example, acetyl-CoA, succinate, pyruvate are the main constituent for the biosynthesis of cell biomass. Sugars are the main recipe for various biosynthesis and gluconeogenesis process is responsible for growth.

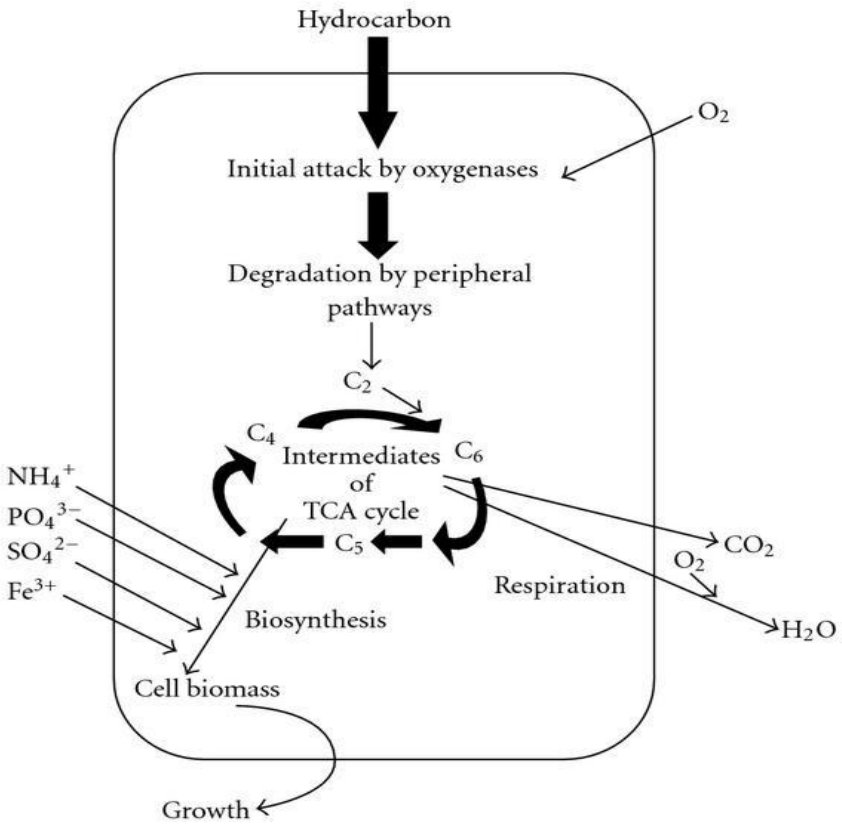

Figure 1: Main principle of aerobic degradation of hydrocarbons by microorganisms [33].

The degradation mechanism of petroleum hydrocarbons can be mediated by certain specified enzyme system. Other mechanisms involved are (1) attachment of microbial cells to the substrates and (2) production of biosurfactants.

Table 1: Enzymes involved in biodegradation of petroleum hydrocarbons.[33]

Enzymes	Substrates	Microorganisms
Soluble Methane Monooxygenases	C_1–C_8 alkanes alkenes and cycloalkanes	*Methylococcus* *Methylosinus* *Methylocystis* *Methylomonas* *Methylocella*
Particulate Methane Monooxygenases	C_1–C_5 (halogenated) alkanes and cycloalkanes	*Methylobacter* *Methylococcus,* *Methylocystis*
Alkane Hydroxylases	C_5–C_{16} alkanes, fatty acids, alkyl benzenes, cycloalkanes and so forth	*Pseudomonas* *Burkholderia* *Rhodococcus,*
Eukaryotic P450	C_{10}–C_{16} alkanes, fatty acids	*Candida maltosa* *Candida tropicalis* *Yarrowia lipolytica*
Bacterial P450 oxygenase system	C_5–C_{16} alkanes, cycloalkanes	*Acinetobacter* *Caulobacter* *Mycobacterium*
Dioxygenases	C_{10}–C_{30} alkanes	*Acinetobacter sp.*

2.7 Uptake of Hydrocarbons by Biosurfactants [33]

Biosurfactants are considered as a heterogeneous group of surface active chemical complex compounds produced by a wide range of microorganisms. Surfactants are the compound which work as solubilization agent and therefore enhances removal of contaminants. Since surfactant

enhances mineralization or solubilization of pollutants thus it also increases biodegradation by increasing the bioavailability of pollutants. Cameotra and Singh reported the bioremediation of oil sludge using biosurfactants. Among bacteria that are capable of utilizing hydrocarbons as carbon and energy sources and producing biosurfactants *Pseudomonas* are the best known. And therefore it is widely studied for the production of glycolipid type biosurfactants. Among Pseudomonas species *P. aeruginosa* are most popular for glycolipid production. However, other species like *P. putida* and *P. chlororaphis* are also reported for producing glycolipid type biosurfactants. Biosurfactants increases the availability of the oil for the utilization of bacteria by increasing its surface area. Summary of the recent reports on biosurfactant production by different microorganisms is given in table: 2.

Table 2: Biosurfactants produced by microorganisms. [33]

Biosurfactants	Microorganisms
Sophorolipids	*Candida bombicola*
Rhamnolipids	*Pseudomonas aeruginosa*
Lipomannan	*Candida tropicalis*
Rhamnolipids	*Pseudomonas fluorescens*
Surfactin	*Bacillus subtilis*
Glycolipid	*Aeromonas* sp.
Glycolipid	*Bacillus sp*.

Biosurfactants can act as an emulsifying agent thus it decreases the surface tension of the organic compounds and help in forming micelles. These micelles are like a microdroplet which can be encapsulated in the hydrophobic microbial cell surface and are taken inside and degraded. In figure: 2 whole mechanisms is demonstrated such as the involvement or role of biosurfactant (rhamnolipids) and the mechanism of formation of micelles and the uptake of hydrocarbon in the form of micelle.

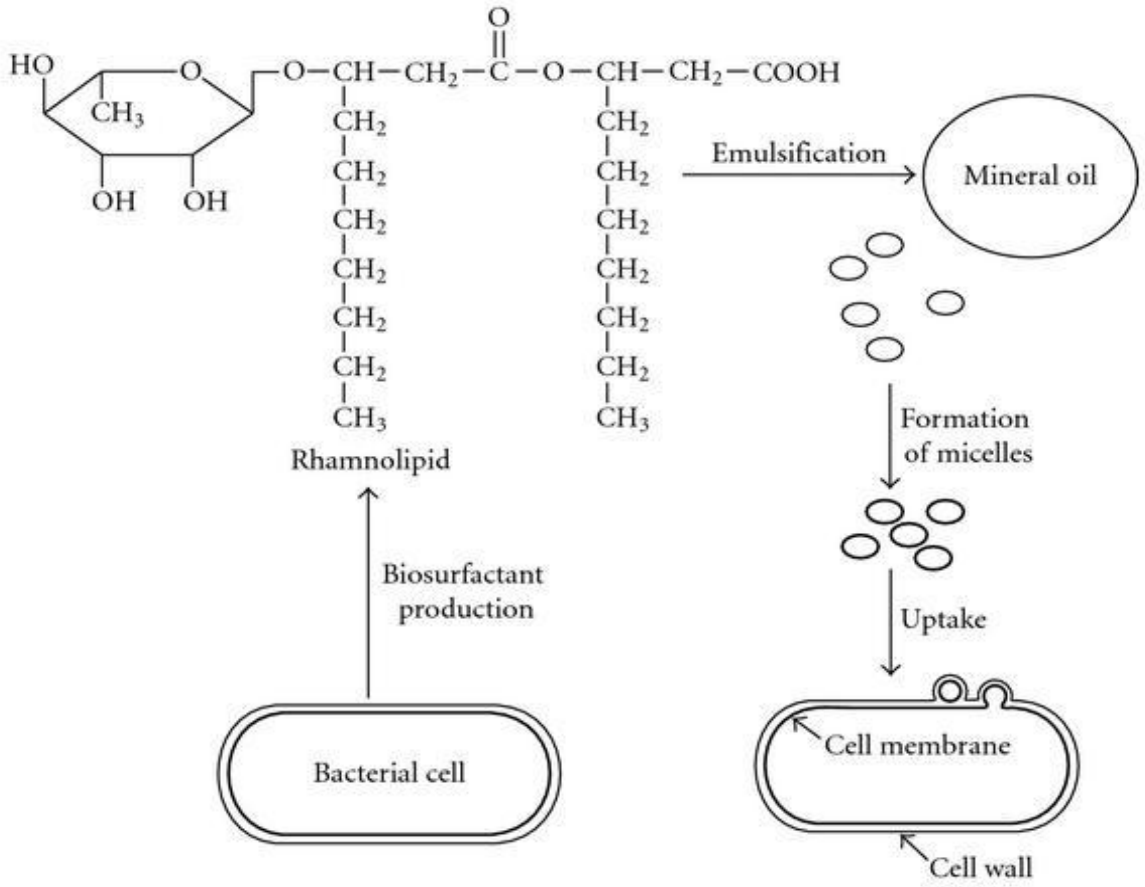

Figure 2: Involvement of biosurfactant (rhamnolipid) produced by *Pseudomonas* sp in hydrocarbons uptake [33].

2.8 Different methods for cleaning of oil contamination.

- **Boom:** This is the primary step to protect the oil contaminants from spreading. In this method oils are bound in a small area by a piece of plastic with a floating cylinder on the top and bottom is weighted so that it floats on a surface with under water "skirt".

  Sorbent booms are made from adsorbing materials that can absorb oil, and are most effective on thin, light oil slicks. While removing the sorbent booms great caution must be taken, so that the adsorbed oil should not squeezed back.

Fig: 3 Oil booms 1. Hard boom, 2. Sorbent boom [37]

- **Skimmer:** Skimmers are boats like structure that are used to skim oil from the water surface. Using a skimmer to remove oil from water is advantageous because it doesn't change the physical or chemical properties of the oil. Skimmers are attached to the settling tanks, There are many factors on which success of skimming depends like type and thickness of the oil spill, the debris amount present in the water, the location of spill and the weather conditions.

  Advantages:

  1. Simple to operate
  2. They are very compact
  3. They will recover most type of oil and emulsions

  Disadvantages:

  1. Work only in calm environmental conditions.
  2. Can recovery upto 80% water.

3. Recovery of high viscosity oil is not easy.

- **Sorbents:** Sorbent materials are also applied to the water surface as powders. It is generally the final step of clean-up of oil spills, as they can absorb trace quantity of oil that could not be skimmed off through skimmer. There are two types of sorbent commonly used natural and synthetic. natural organic sorbent materials, such as peat moss and sawdust, or synthetic organic sorbent materials, such as polypropylene, polyester foam or polystyrene. Sorbents are generally applied manually, and recovered with the use of nets and rakes.
- **In-situ Burning:** This is a method of cleaning of oil spill and is used to remove oil from the surface of the water. It is the post skimmer process it is applied after oil skimmed off by the skimmer. It causes acid rain due to the burning of oil releases nitrogen and sulphur in the atmosphere. Thus it causes additional pollution while burning the oil from the water surface. It removes oil quickly and efficiently from the surface of water.

  Advantages:

  1. Waste storage and disposal requirements is reduces.
  2. 95% efficiency with minimal equipment and manpower to remove oil.
  3. Mostly lower weight aromatic hydrocarbons removed which are the more toxic and bioavailable components of crude oil.

  Disadvantages:

  1. Not removing oil from environment; trading one form of pollution for another, means it can cause acid rain.
  2. Smoke plume is unpleasant and contains fine particulate matter, PAH's, and other chemicals.

3. Burning is complicated for emulsion.
4. Burning is risky for response personnel.
5. Burn residuesmay effect benthic environment after sinking.
6. Ignition of oil is quite difficult.

Fig: 4 In- Situ burning of oil spill [37]

- **Chemical Dispersants:** Chemical dispersants is another method of cleaning up oil spills. Dispersants are chemicals compounds that are applied to the surface of the water where oil spill has occurred, usually by a low-flying plane. Oil can eventually break down naturally, and chemical dispersants help to speed up the natural process. Dispersants main work is to bind to the oil and move the oil further down the water column, that means the oil disperses into the water. Thus water dilutes the oil to a concentration level that is less harmful to aquatic flora and fauna near that region of spill.

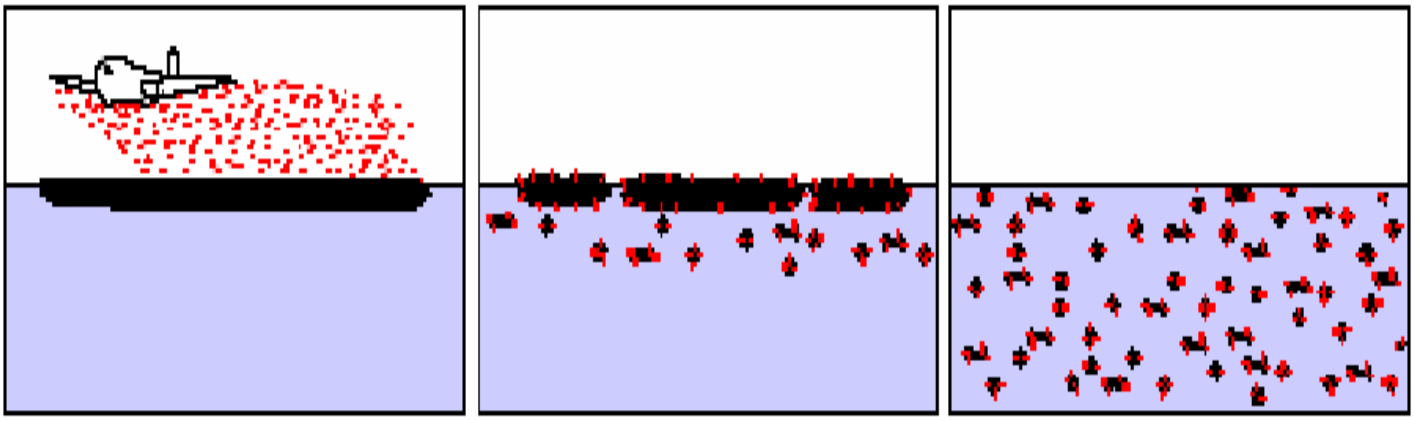

Fig: 5 Application of Chemical Dispersants to an Oil Spill [37].

Advantages:

1. This method prevented oil from moving into sensitive environments or spreading onshore. Therefore reduces damage to important coastal flora and fauna.
2. After treating with dispersant small chemical droplets do not form tar ball and patties.
3. It reduces risk of death of birds & mammals due to hypothermia, because oil interferes with animal's ability to maintain body temperature, thus causing death. Since after dispersing oil do not have capability to interfere
4. Oil concentrations limits the overall impact to sensitive environmental resources due to it is extremely low and dilute quickly in ocean.

Disadvantages:

1. Dispersion process main drawback is that it exposes water column & near shore shallow bottom-dwelling organisms to oil as due to dispersion oil moves from surface to water column.
2. Dispersants and dispersed oil particles are toxic to some of the habitat of marine.

3. Effectiveness is totally dependent on the type of oil contaminants and on the environmental condition.
4. Energy required for the dispersion as it is very essential to dissolve the dispersant with oil and water.
5. On heavier oil success rate of dispersion is low.

- **Bioremediation:** Microorganisms can effectively remove contaminants from the water and soil. The microorganisms can break down many harmful chemicals, including gasoline and oil. Bioremediation is a process that naturally occurs, to some extent, after every oil spill. Bioremediation is applied after the majority of the oil spill is cleaned up manually, biological processes help by breaking the trace amounts of organic compounds that could not be removed manually. Biological processes, together with natural processes such as evaporation, oxidation, weathering, causes the breaking down of the oil and naturally clean up the environment.

Advantages:

1. Bioremediation is very efficient process since bacteria consumes oil till there is no residue left.
2. Bioremediation agents help in activating bacteria that is already present at the site.
3. Helpful in cleaning of oil contamination at the site where there is scarcity of the nutrients such as (Oxygen and Nitrogen)
4. Bacteria do not causes any harmful effect on the vicinity environment as it expires itself once it consumes the oil at the site.
5. Microorganism rapidly increase cause faster removal of the contaminants.

Disadvantages:

1. Biodegradation is a sluggish process & in case of heavy oils it often remains incomplete.
2. For a oil spill is it is not a appropriate process due to large volume of oil is present.
3. It cannot be an ultimate process for cleaning of oil spill it is a finishing technique only to remove residual oil.
4. Since for the removal of oil it is necessary that it should be available to the microorganism therefore it only works properly in calm water.

- **In-Situ Chemical Oxidants:** Chemical oxidation is a technique to treat contaminated soil and groundwater systems. Chemical oxidants work by oxidizing organic contaminants. Chemical oxidants perform a Physico-chemical process which destroy the persistant contaminated and is not affected by environmental and other factors. Some examples are:

  i. Hydrogen Peroxide and Fenton reagent.
  ii. Permanganate
  iii. Ozone
  iv. Sodium Persulfate etc.

Advantage:

1. It is a fast process because time require for treatment is less.
2. It can treat concentrated contaminant
3. Effective for a diverse range of contaminants.

Disadvantage:

1. Oxidation process is non selective
2. For proper oxidation pH, temperature and contact time should be monitor for desired extent of oxidation.
3. Implementation cost is quite high.

- **Activated carbon:** Activated carbon is a well known adsorbent. Activated carbon can also be used for adsorbing Poly Aromatic Hydrocarbons. Adsorption of PAH on activated carbon mainly depends on their porous texture.

Advantages:

1. PAH can be regenerated after adsorbing on activated carbon.
2. Activated carbon can adsorb high molecular weight PAH.
3. Its PAH adsorbing capacity is very high more than 90%.

Disadvantages:

1. It is costly adsorbent as compared to biological fiber.
2. Adsorption time is more as compared to biological fiber.
3. Availability is less as compared to biological fibers.

CHAPTER - 3

MATERIALS AND METHODS

3.1 Petroleum sludge collected from the industry (HPCL, Vishakhapatnam) is analyzed to estimate the petroleum hydrocarbon concentration in the effluent. About 10% of the effluent was used for adsorption study.

Table: 3 Characteristic parameters of sludge

Sludge liquor characteristics	**Unit**	**values**
pH	–	5.8
Temperature	ºC	25
Specific gravity	–	0.91
DO	mg/l	3.7
Conductivity	Scm-1	19
THC	mg/l	126

3.2 Bacterial strain

Pseudomonas aeruginosa is used as a microbe to degrade petroleum products. Its optimum growth condition was observed as temp:- 35^0c - 42^0c , pH:- 6- 9 and salinity:- 2%-12%.

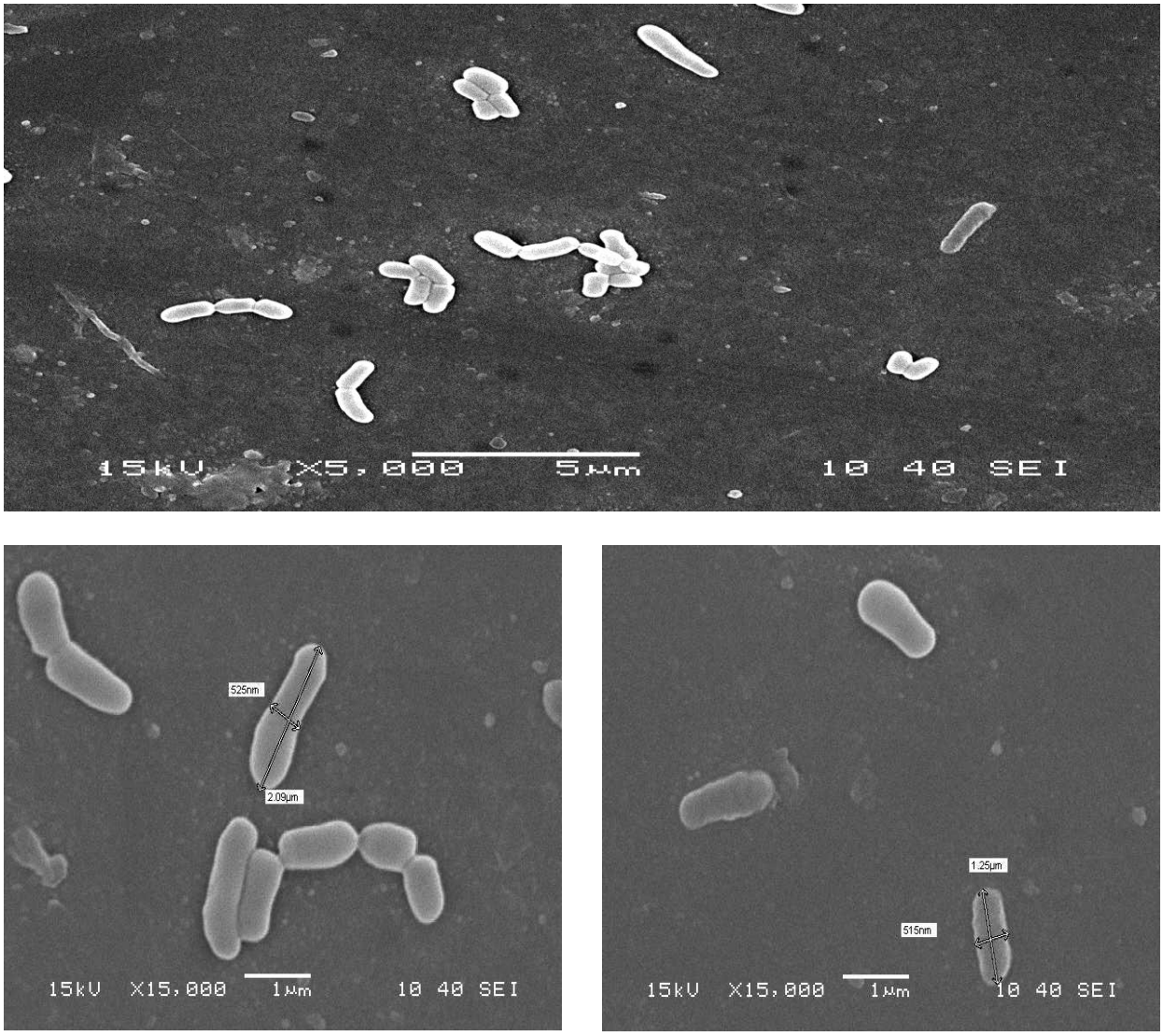

Fig: 6 Scanning electron microscope of Pseudomonas aeruginosa

3.3 Nutrient media

1. Composition of MS media used to culture the microorganism:

Agar	-	15 gm/l
Peptone	-	5.0 gm/l
NaCl	-	5.0 gm/l
Beef extracts	-	1.5 gm/l

Yeast extracts - 1.5 gm/l

2. Mineral salt media used during experiments.

Chemical	**wt/ l**
KH_2PO_4	0.7 g
Na_2HPO_4	0.9 g
$NaNO_3$	2 g
$MgSO_4.7H_2O$	0.4 g
$CaCl_2.2H_2O$	0.1 g

2 ml of trace elements (per liter)

$FeSO_4.7H_2O$	2 g
$MnSO_4.H_2O$	1.5 g
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.6 g

Additional chemical (only for rhamnolipid assay)

CTAB	200 μg/ml
Methylene blue	5 μg/ml
Agar	1.5%

3.4 Biological fiber

In this experiment coconut coir and jute fiber is used as biological fiber. The Fibers were washed thoroughly with distilled water to remove the surface adhered particles and water-soluble matter. It subsequently was spread on trays and oven dried at 70^0 C for 48 h to remove all the moisture content.

Table: 4 Compositions of Fibers [34]

Fibers	**Hemi cellulose**	**Cellulose**	**Lignin**
Coconut	38%	28%	32.8%
Jute	20%-24%	58%-63%	12%-15%

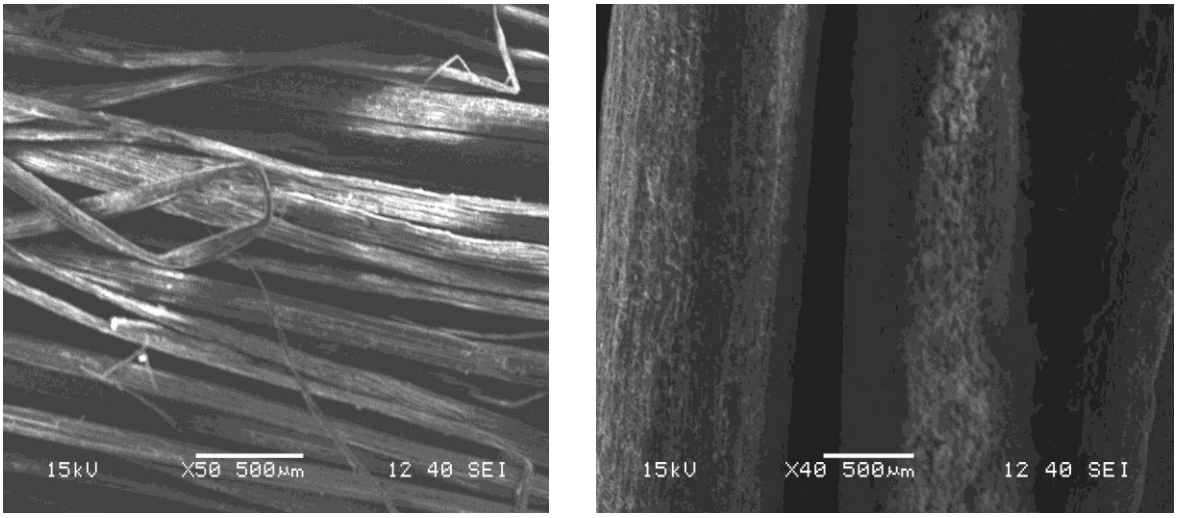

Fig: 7 Scanning electon microscope of 1. Jute fiber 2. Coconut fiber

3.5 Analysis of growth of the culture was done by taking biomass reading by using UV-Visible Spectrophotometer at 600 nm.

3.6 GC-MS analysis of petroleum sludge samples was done at SARGAM LABORATORY PVT LTD, Chennai,

3.7 Taguchi Method [35]

Parameter optimization is done by using Taguchi experimental design method. Taguchi is very simple designing method and it offers a systematic approach for optimization of various parameters for better performance, best quality and low cost. And number of experiments is also reduced, as a result both time and cost of experiment is saved. Taguchi method plans the experiment in terms of orthogonal array that find out different combinations of parameters and their levels for each experiment. Main effect analysis is performed on the basis of average output value of the quality characteristic at each parameter level. After that ANOVA is determine which helps in find out that which process parameters is statistically significant and its contribution. With the help of main effect and ANOVA analyses, possible combination of optimum parameters can be predicted. Finally, an experiment is conducted to verify the optimal process parameters obtained from the process parameter design. Taguchi method uses the signal/noise ratio to measure the quality characteristic deviation from the desired value. The experimental conditions having the maximum S/N ratio are considered as the optimal conditions, and the variability characteristics are inversely proportional to the S/N ratio.

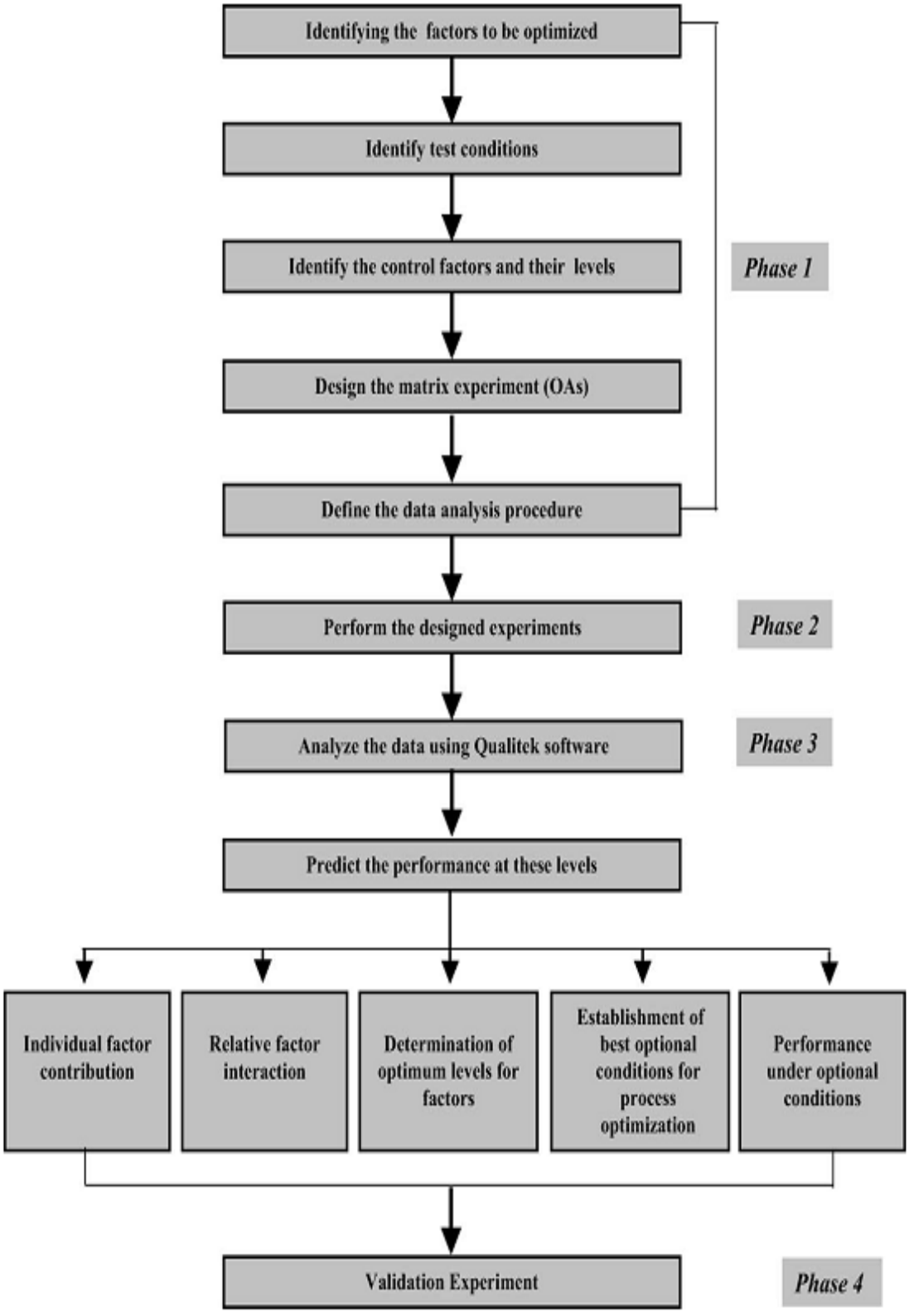

Fig: 8 Schematic representation of the steps involved in the Taguchi DOE methodology designed for optimization[35].

Design of experiments: Based on literatures operational parameters and their levels were selected and showed in Table 5. The orthogonal array of L_{16} type was used. L and 16 mean Latin square and the replication number of the experiment, respectively. Three–four level factors can be positioned in an L16 orthogonal array table. The number in column indicates the levels of a factor.

Table: 5 Parameters and their values corresponding to their levels to be studied in experiments.

PARAMETERS	Level**1**	Level**2**	Level**3**	Level**4**
A. Temperature	20	30	40	50
B. pH	6	7	8	9
C. Nitrogen source	NH_4NO_3	$NaNO_3$	$(NH_4)_2SO_4$	Peptone

CHAPTER – 4

RESULT AND DISCUSSION

4.1 ADSORPTION STUDY OF THE FIBERS

The adsorption study of the fiber is performed in a fixed bed column. The experiments were performed using different amount (by weight) combination of coconut fiber and jute fiber. About nine different experiments were performed using different combination of fibers at different weight (2 gm - 6 gm). These experiments were performed at the temperature 25^0c, pH 6.5 and 10% sludge water solution. In this experiment 10% sludge solution was passed through the column that consisted of biological fiber bed. After single run the fiber was taken out and oven dried for 40^0c for 24 hrs in order to remove all water contents from the fiber and was weighed. The increased in weight of the used fiber evidenced the adsorption of petroleum sludge.

Different combination of fibers: In this study nine experiments have been conducted at different combination of fibers by weight ratio.

Table: 6 Different combinations of fibers for adsorption.

No. of Experiment	Jute fiber : Coconut fiber	After adsorption (oven dried for 24 hour at 40^0c) in gms
1	1gm : 1gm	3.177 : 4.505
2	1gm : 2gm	3.167 : 7.054
3	1gm : 3gm	3.161 : 11.085
4	2gm : 1gm	7.501 : 4.465
5	2gm : 2gm	9.049 : 9.564
6	2gm : 3gm	3.054 : 11.872
7	3gm : 1gm	11.378 : 4.352
8	3gm : 2gm	11.524 : 6.658
9	3gm : 3gm	7.308 : 11.400

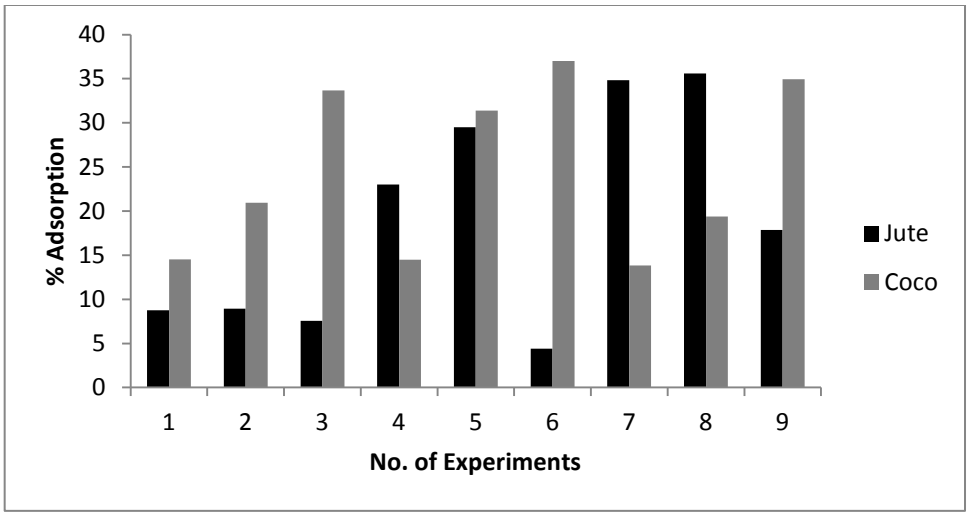

Figure: 9. % adsorption of fiber of different combination.

From the above figure: 5 it is clear that experiment no.5 i.e. combination 2:2 show maximum adsorption therefore this combination is considered in further experiment.

4.2 SEM OF FIBERS BEFORE AND AFTER TREATING WITH PETROLEUM SLUDGE

1. COCONUT FIBER

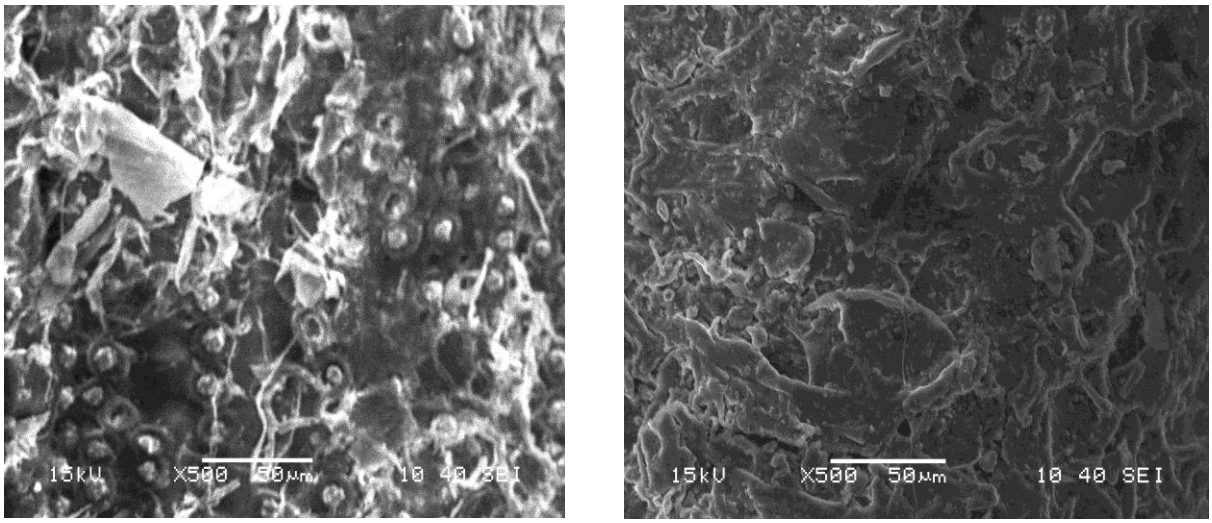

Fig: 10 Scanning electron microscope of: 1) Coconut fiber without petroleum sludge adsorption. 2) Coconut fiber after petroleum sludge adsorption.

2. JUTE FIBER

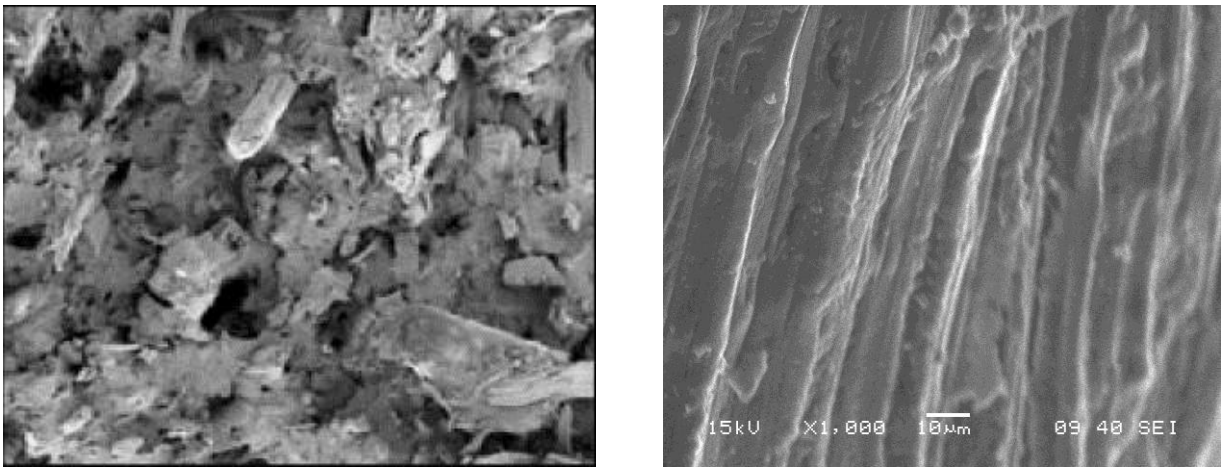

Fig: 11 **Scanning electron microscope of: 1) Jute fiber without petroleum sludge adsorption. 2) Jute fiber after petroleum sludge adsorption**

SEM analysis of biological fibers before and after petroleum treatment was done and it is illustrated in the figure 7 and 8. From the figure 7 and 8 it is clear that the surface of fiber changed after the treatment with petroleum sludge. It is visible that a layer of petroleum hydrocarbon is on the surface of the treated fiber. In order to prove this an elemental analysis of fiber was also done and its result is given below.

4.3 EDX RESULT OF FIBER BEFORE AND AFTER TREATING WITH PETROLEUM SLUDGE

Elemental analysis of biological fiber (coconut and jute) was done to find out the elemental changes that occur on the surface of fiber after the treatment with the petroleum sludge. This analysis reflects elemental changes occur on the surface of the fiber. For this analysis four samples were taken: 1. Coconut fiber without treatment 2. Jute fiber without treatment 3.Coconut fiber with petroleum sludge treatment and 4.Jute fiber with petroleum sludge treatment.

1. **COCONUT FIBER**

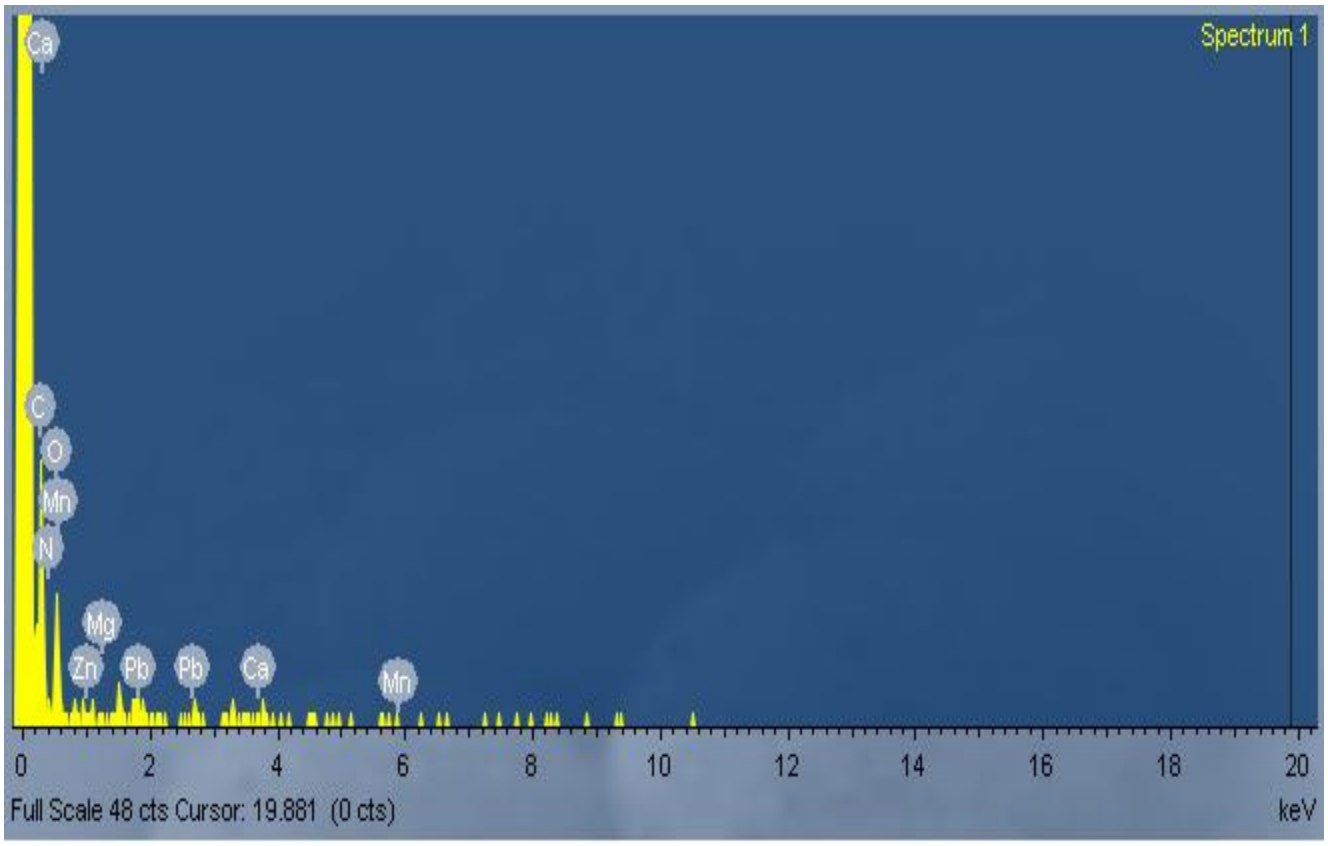

Fig: 12 EDX graph of non treated coconut fiber.

Table: 7. EDX of non treated coconut fiber

Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C	99.67	1.4151	33.88	10.69	38.16
N	17.24	0.2235	37.13	15.30	35.86
O	31.31	0.4863	30.98	11.98	26.19
Mg	0.90	0.8770	0.49	1.62	0.28
Ca	0.18	0.9532	0.09	3.20	0.03
Mn	0.00	0.7452	0.00	9.48	0.00
Zn	-2.41	0.4735	-2.45	4.63	-0.51
Pb	-0.21	0.7442	-0.14	6.87	-0.01
Cr	0.00	0.7245	0.00	4.56	0.00
Totals			100.00		
Element	App	Intensity	Weight%	Weight%	Atomic%

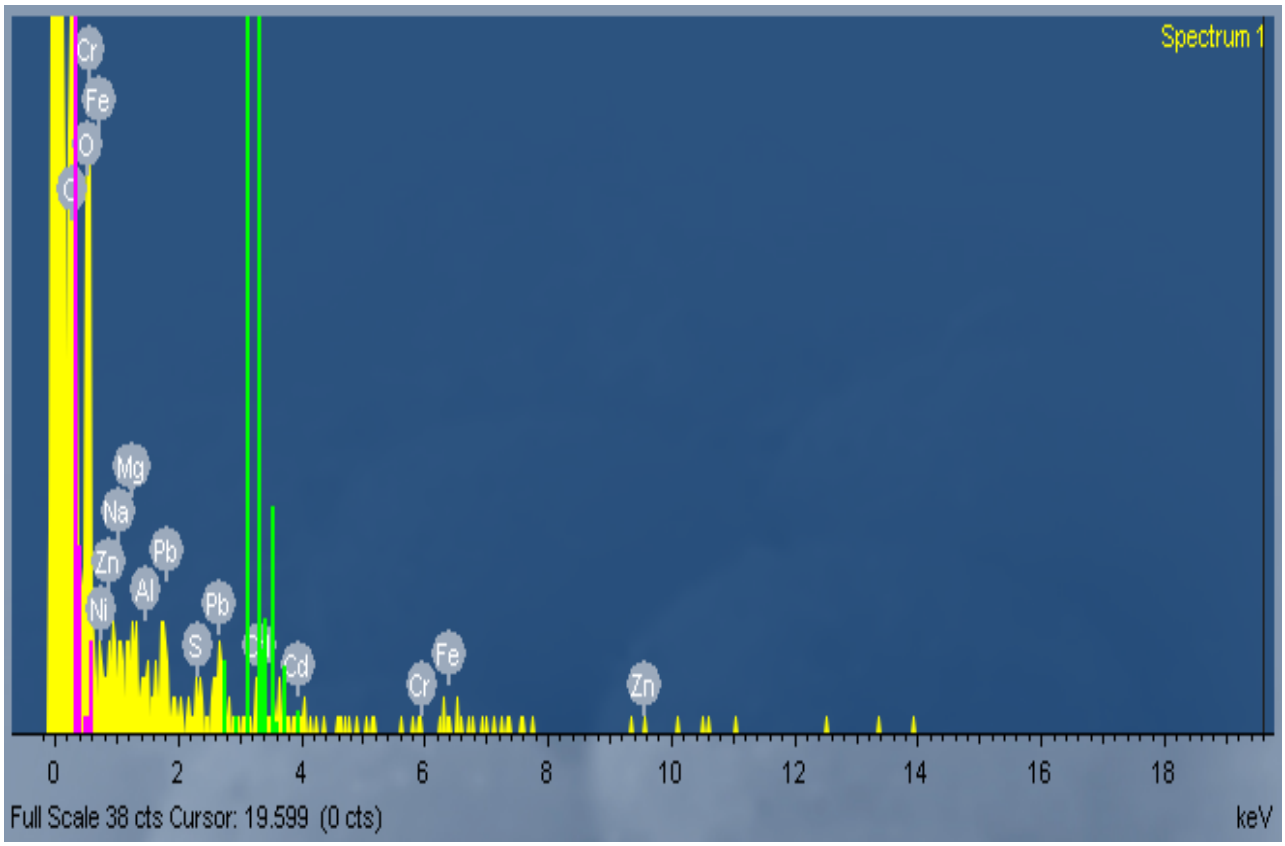

Fig: 13 EDX graph of petroleum adsorbed coconut fiber.

Table: 8. EDX of Petroleum treated coconut fiber

Element	App	Intensity	Weight%	Weight%	Atomic%
	Conc.	Corrn.		Sigma	
C	66.86	1.1996	55.23	11.19	61.43
O	34.70	0.7601	45.23	10.02	37.76
Na	0.92	1.0338	0.88	2.18	0.51
Mg	0.87	0.8818	0.98	1.20	0.54
Al	0.15	0.9257	0.16	0.81	0.08
S	1.23	0.9617	1.26	1.12	0.53
Cr	0.01	0.7650	0.01	4.21	0.01
Fe	-1.51	0.7553	-1.99	6.66	-0.48
Ni	0.03	0.7549	0.03	11.10	0.01
Zn	-0.83	0.4853	-1.69	4.65	-0.35
Cd	-0.36	0.7201	-0.50	3.95	-0.06

1. JUTE FIBER

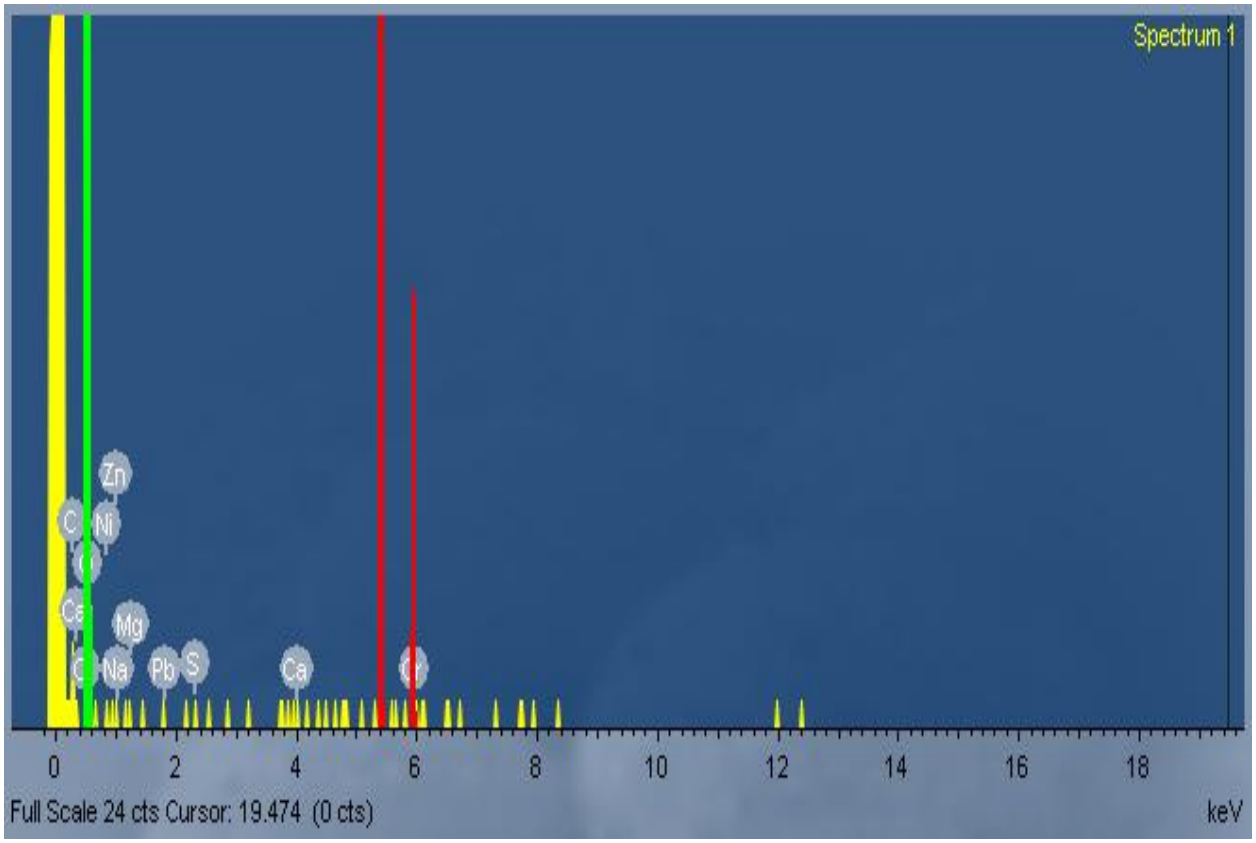

Fig: 14 EDX graph of non treated Jute fiber.

Table: 9. EDX of non treated Jute fiber.

Element	App	Intensity	Weight%	Weight%	Atomic%
	Conc.	Corrn.		Sigma	
C	80.28	1.3558	30.41	11.46	35.37
N	14.36	0.2422	30.46	15.42	30.38
O	43.20	0.5676	39.10	14.71	34.14
Na	0.50	0.9834	0.26	2.68	0.16
Mg	0.14	0.8578	0.08	1.52	0.05
Cr	0.00	0.7668	0.00	6.92	0.00
Fe	0.01	0.7566	0.00	10.71	0.00
Ni	0.01	0.7562	0.01	18.25	0.00
Zn	-0.53	0.4609	-0.59	5.62	-0.13
Pb	0.36	0.7436	0.25	6.29	0.02

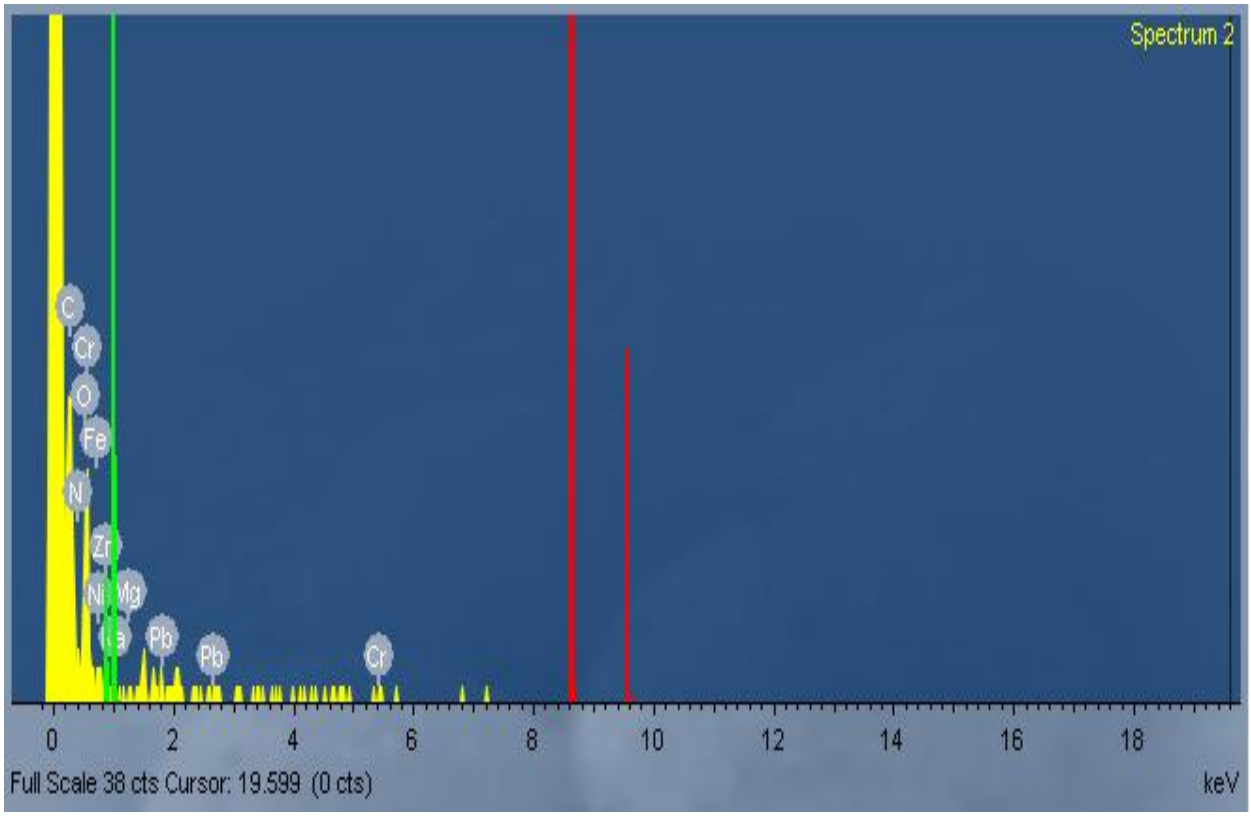

Fig: 15 EDX graph of petroleum adsorbed Jute fiber.

Table: 10. EDX of Petroleum treated Jute fiber.

Element	App	Intensity	Weight%	Weight%	Atomic%
	Conc.	Corrn.		Sigma	
C	88.96	1.6514	33.40	59.93	37.03
N	23.57	0.2278	64.18	104.22	61.01
O	1.18	0.3134	2.33	84.12	1.94
Na	0.00	1.0792	0.00	12.89	0.00
Mg	0.00	0.9197	0.00	7.48	0.00
S	0.00	0.9704	0.00	9.47	0.00
Ca	0.01	0.9499	0.00	15.53	0.00
Cr	0.01	0.7597	0.01	36.70	0.00
Fe	0.02	0.7489	0.02	56.88	0.00
Ni	0.04	0.7476	0.03	97.03	0.01
Zn	0.01	0.5072	0.01	26.69	0.00

Above table 7-10 and figure 12-15, shows the elemental analysis of fibers surface before and after the petroleum treatment and from the result it is clear that jute fiber and coconut fiber surface changed after the petroleum sludge treatment, table 7, 8, 9 and10 shows that surface element density increases after the treatment with petroleum sludge. In the table 7-10 elemental increase is clearly visible such as in coconut fiber there is increase in chromium, sulphur, aluminum, nickel etc. and similarly in jute fiber there is increase in element such as zinc, nickel, iron, chromium etc. therefore it is concluded that coconut and jute fiber has the ability to adsorb the petroleum hydrocarbons and elements present in the petroleum sludge.

4.4 FTIR RESULT OF BIOLOGICAL FIBER BEFORE AND AFTER TREATING WITH PETROLEUM SLUDGE

1. COCONUT FIBER

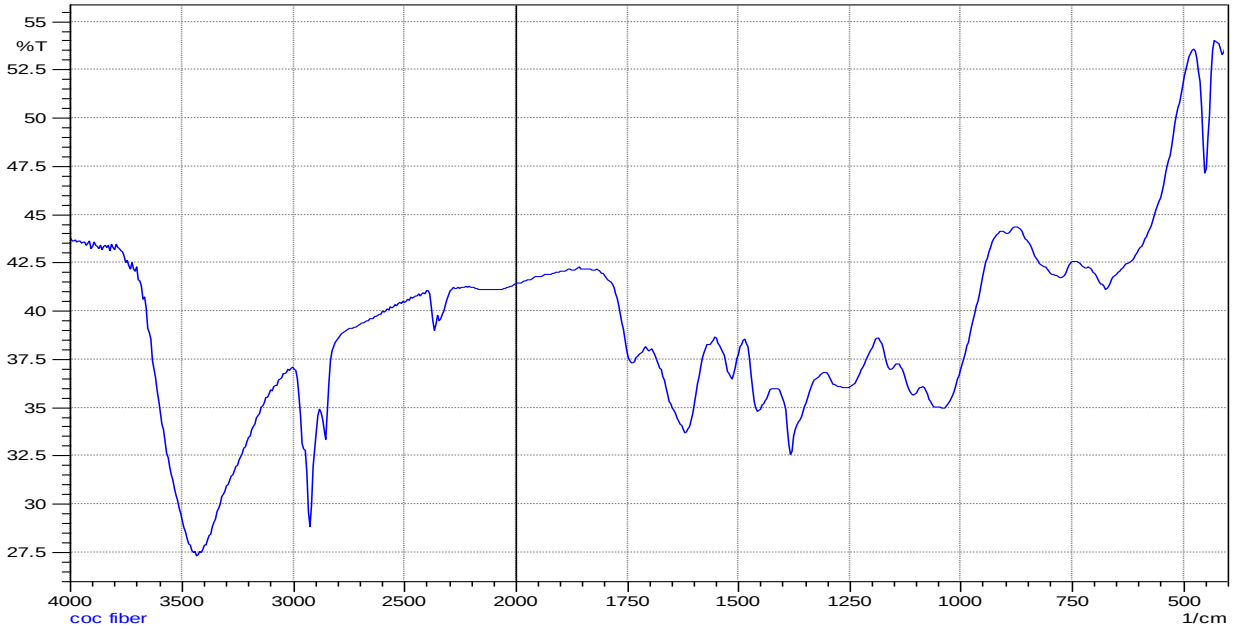

Fig: 16. FTIR result of coconut fiber before treating with petroleum.

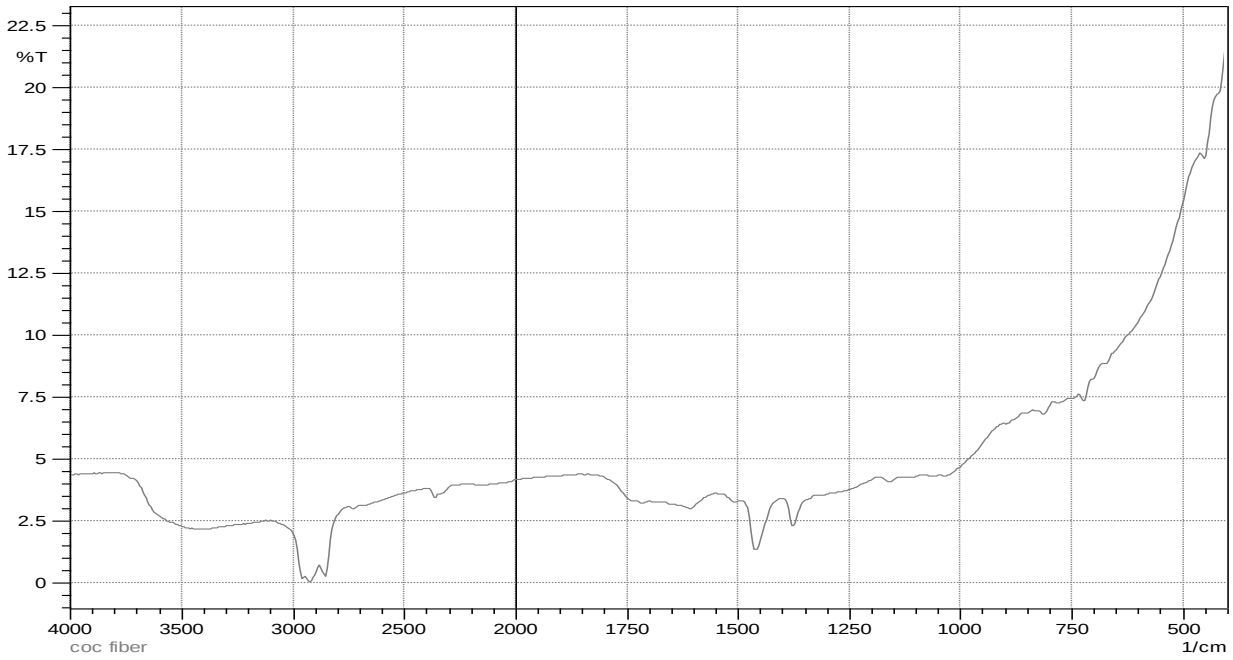

Fig: 17. FTIR result of coconut fiber after treating with petroleum.

2. JUTE FIBER

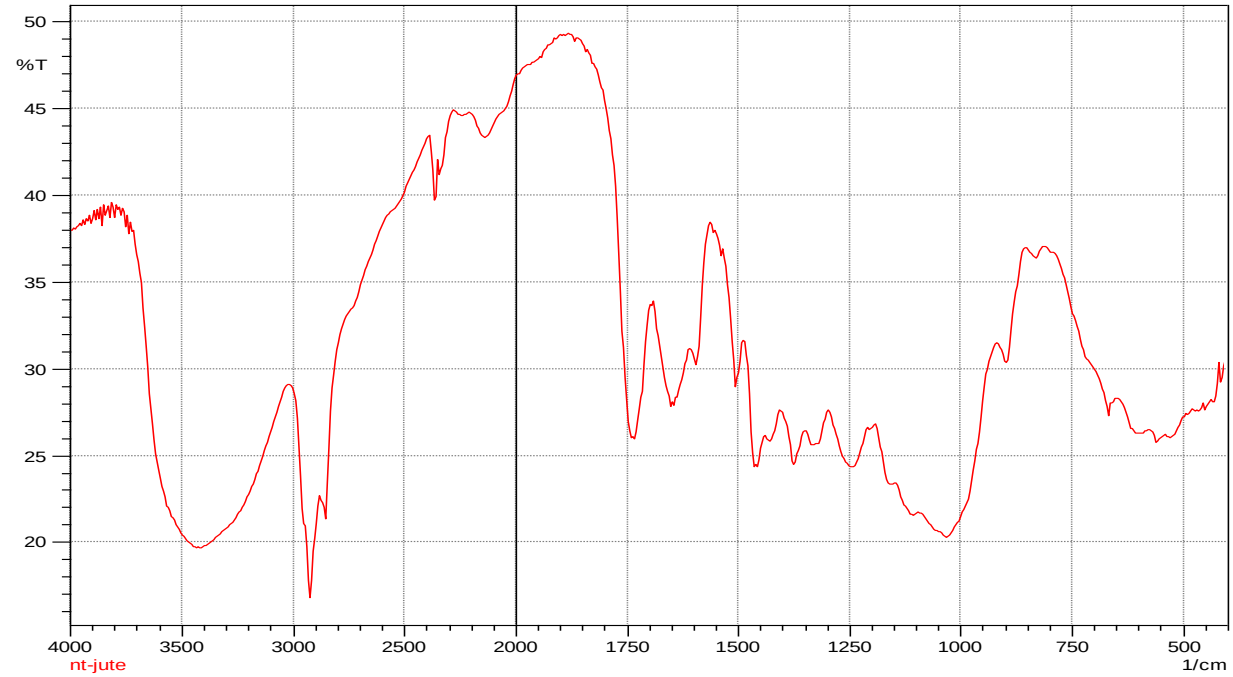

Fig: 18. FTIR result of jute fiber before treating with petroleum.

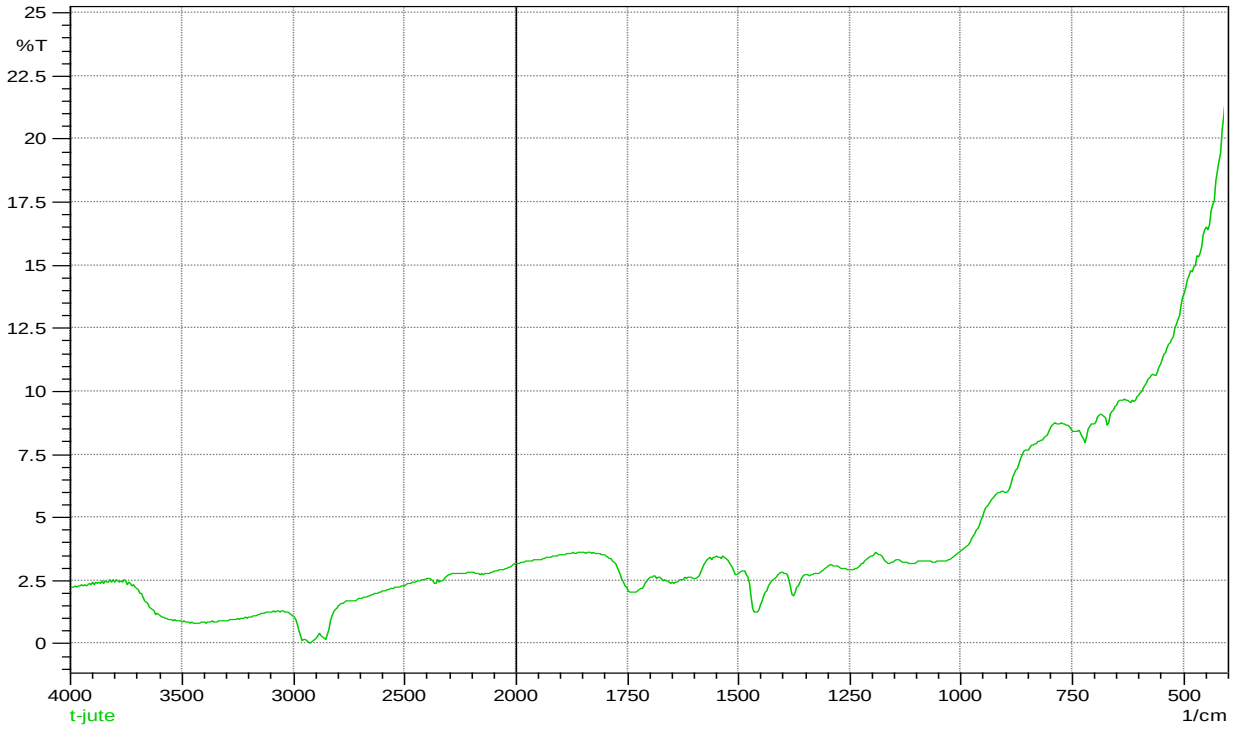

Fig:19. FTIR result of jute fiber after treating with petroleum.

Table: 11 FTIR result shows functional group presence in treated and non treated fiber [36].

Wavenumber (cm^{-1})	**Functional group**	**Non treated coconut fiber**	**Treated coconut fiber**	**Non treated jute fiber**	**Treated jute fiber**
2970-2950, 2880-2860, 1470-1430, 1380-1370, 1385-1380, 1370-1365, 1395-1385	Methyl ($-CH_3$)	Gem-dimethyl	**Methyl C-H asym/sym stretch, Methyl C-H asym/sym bend**	Methyl C-H asym/sym bend	Methyl C-H asym/sym bend,
2935-2915, 2865-2845, 1485-1445, 750-720, 1055-1000,	Methylene ($>CH_2$)	Cyclohexane ring, Methylene C-H bend, Methylene	Methylene C-H asym/sym stretch, Methylene C-H bend,	Methylene C-H asym/sym stretch, cyclohexane ring	Methylene C-H asym/sym stretch, cyclohexane

1005-925		C-H asym/sym stretch	Cyclohexane ring, **Methylene -$(CH_2)_n$-**		ring vibration, **Methylene -$(CH_2)_n$- Methylene C-H bend**
2900-2880, 1350-1330, 1300-700,	Methyne (>CH-)	Methyne C-H stretch	Methyne C-H stretch, **Methyne C-H bend, Methyne C-H asym/sym stretch, skeletal C-C vibration**	Methyne C-H bend	Methyne C-H bend, **Methyne C-H asym/sym stretch, skeletal C-C vibration**
1680-1620, 3095-3075, 1420-1410, 995-985, 970-960	Alkene	Alkene C=C stretch	Alkene C=C stretch, **Aryl substituted C=C,Conjugate C=C**		
1615-1580, 1510-1450, 3130-3070, 1225-950, 900-670	Aromatic ring		**Aromatic ring stretch, Aromatic C-H in plane bend, Aromatic C-H out of plane bend.**	Aromatic ring stretch, Aromatic C-H out of plane bend	Aromatic ring stretch, Aromatic C-H out of plane bend, **Aromatic C-H stretch, Aromatic C-H in plane bend**

2140-2100, 2260-2190, 3320-3310, 680-610	Alkyne		**Alkyne C-H bend, medival alkyne, Alkyne C-H bend**	Terminal alkyne	Terminal alkyne, **Alkyne C-H stretch, medival alkyne, Alkyne C-H bend**
1150-1000, 800-700, 700-600, 600-500	Aliphatic organohalogen		**Aliphatic fluro, chloro, bromo and iodo compound**	Aliphatic fluro, bromo and iodo compound	Aliphatic fluro, bromo, iodo and **chloro compound**
3570-3200, 3400-3200, 3550-3450, 3570-3540, 3675-3600, 3645-3630, 3635-3620, 3620-3540, 3640-3530	Alcohol compound (O-H)	Normal Polymeric O-H stretch	polymeric OH stretch, **Dimeric O-H stretch**	Hydroxy group, normal polymeric OH stretch	Hydroxy group, normal polymeric OH stretch, **Dimeric O-H stretch**
1050, 1100, 1150, 1200	(C-O)		**1^0, 2^0, 3^0 Alcohol, Phenol.**		**1^0, 2^0, 3^0 Alcohol, Phenol.**
2820-2810, 1150-1050, 1140-1070, 1270-1230, 890-820	Ether &Oxy group	Aromatic ether	**alkyl-substitute ether, C-O stretch, cyclic esters, peroxides,**	Aromatic ether, aryl -O- stretch, alkyl-substitute ether, C-O stretch, cyclic esters.	Aromatic ether, aryl -O- stretch, alkyl-substitute ether, C-O stretch, cyclic esters, **Methoxy C-H stretch, peroxides,**

3400-3380, 3345-3332, 3510-3460, 3415-3380, 1650-1590, 1090-1020, 3360-3310	Amine & Amino group	Aliphatic 1^0 amine, N-H stretch	**1^0 amine NH bend, 1^0 amine CN stretch**	Aliphatic 1^0 amine, 1^0 amine CN stretch, 1^0 amine NH bend	Aliphatic 1^0 amine, 1^0 amine CN stretch, 1^0 amine NH bend, **Aliphatic 1^0 amine NH stretch, Aromatic 1^0 amine NH stretch, Aliphatic 2^0 amine NH stretch, 1^0 amine CN stretch**

FTIR analysis of biological fiber was done to find out the functional group presence on the surface of fiber before and after treating with petroleum sludge. From the above Figure 16, 17, 18 and 19 it is clear that there is increase in number of functional group on the surface of treated biological fiber. In the table: 11 functional group present on the surface of fiber after and before the petroleum treatment is compared and it is investigated that number of functional group increases and is highlighted, it is observed that number of functional group Methyl, Methylene, Methyne, Alkene, Alkyne, Aromatic ring, Aliphatic Organohalogen, alcohol, Ether/Oxy, Amine/Amino and Carbonyl group increases on the surface of treated Coconut and jute fibers Therefore from above result it was concluded that hydrocarbon adsorption occurs on the surface of the fibers.

4.5 SCREENING OF RHAMNOLIPID

Pseudomonas aeruginosa, were screened for rhamnolipid production using CTAB-methylene blue indicator plates. *Pseudomonas aeruginosa* were initially assayed for rhamnolipid production using the mineral salt-CTAB-methylene blue agar plate method. Bacteria were grown for 7 days in mineral salt-CTAB-methylene blue agar plate medium under appropriate growth conditions and checked plate periodically. A positive reaction for rhamnolipids is the formation of a purple-blue haze with a sharply defined edge around the culture as shown in figure: 20.

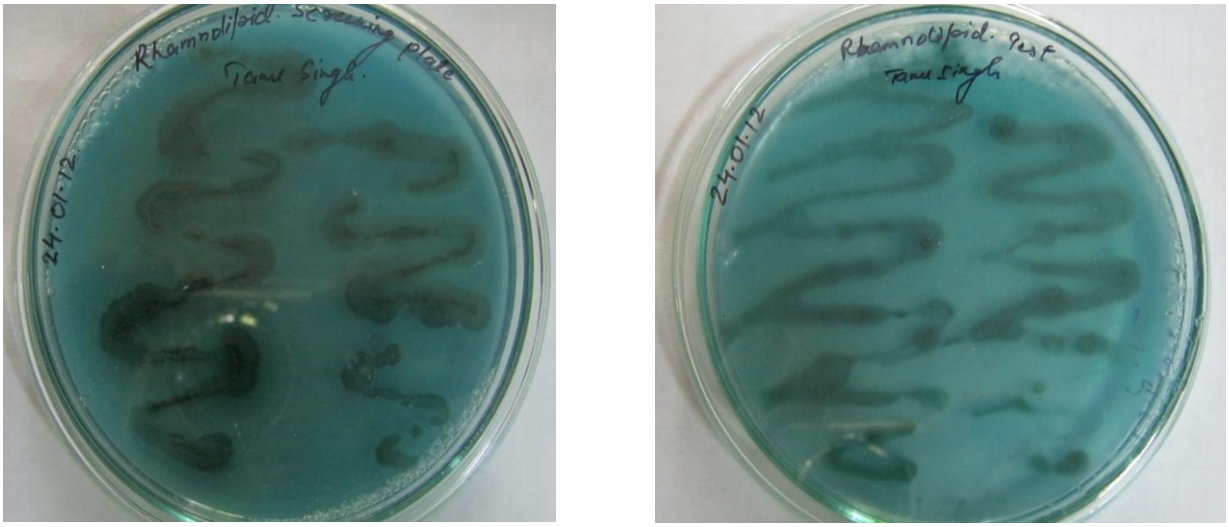

Fig: 20 Rhamnolipid screening plate

4.6 PARAMETER OPTIMIZATION USING TAGUCHI METHOD

In the Taguchi method, the terms ‘signal’ and ‘noise’ represent the desirable and undesirable values for the output characteristic, respectively. Taguchi method uses the S/N ratio to measure the quality characteristic deviating from the desired value. According to Taguchi’s orthogonal array, in this study sixteen experiments were performed and results are presented given in Table: 7.

Table:12. Experimental layout using the L16 orthogonal array and biomass growth results.

Exp no.	Temperature	pH	Nitrogen source	Biomass	S/N ratio
1.	20	6	$(NH_4)_2SO_4$	0.2755	-11.1975
2.	20	7	NH_4NO_3	0.300	-10.4576
3.	20	8	Peptone	0.5645	-4.9667
4.	20	9	$NaNO_3$	0.4411	-7.1093
5.	30	6	NH_4NO_3	0.6682	-3.5019
6.	30	7	$(NH_4)_2SO_4$	1.0199	0.1712
7.	30	8	$NaNO_3$	0.7828	-2.1270
8.	30	9	Peptone	1.1280	1.0462
9.	40	6	Peptone	1.0931	0.7732
10.	40	7	$NaNO_3$	0.5305	-5.5063
11.	40	8	$(NH_4)_2SO_4$	0.9300	-0.6303
12.	40	9	NH_4NO_3	0.7786	-2.1737
13.	50	6	$NaNO_3$	0.1900	-14.4249
14.	50	7	Peptone	0.6300	-4.0132
15.	50	8	NH_4NO_3	0.2333	-12.6417
16.	50	9	$(NH_4)_2SO_4$	0.2000	-13.9794

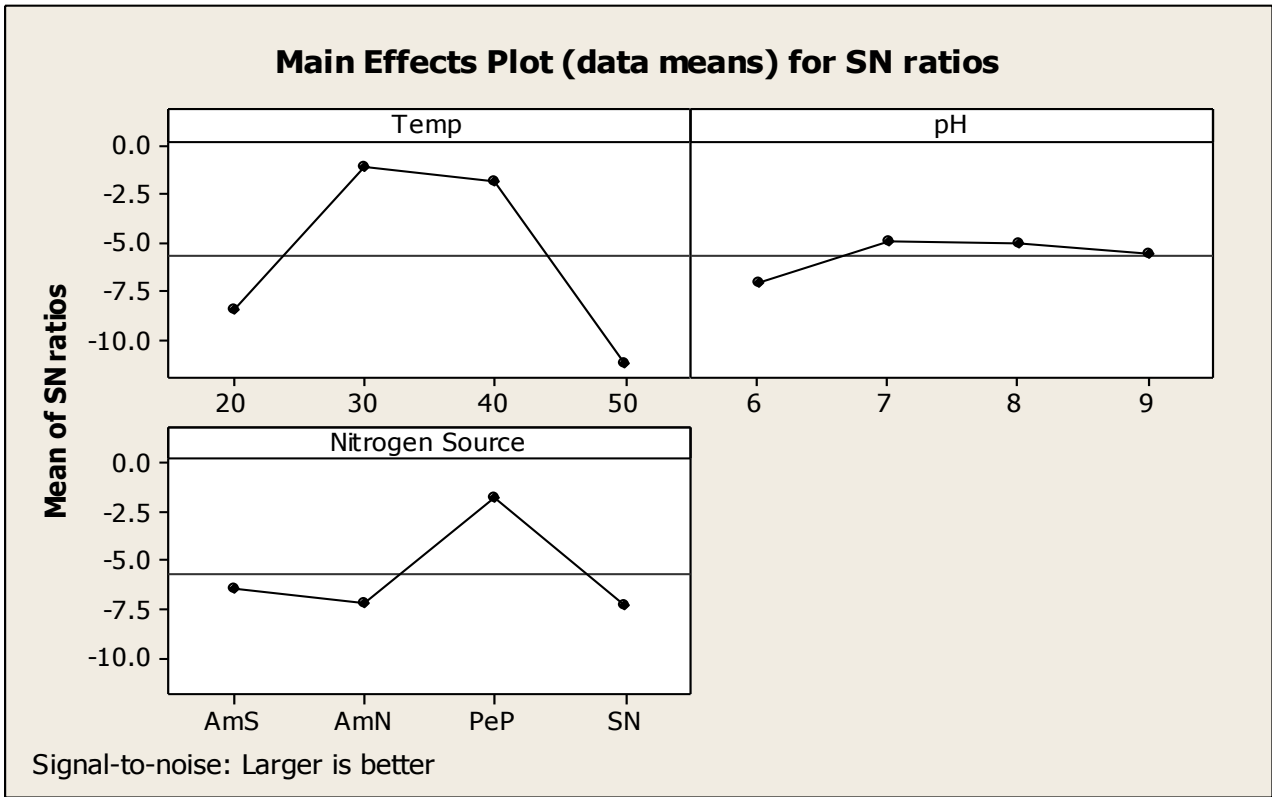

Fig: 21. S/N ratio graph for different parameter

Above figure: 21 is the result of the Taguchi analysis of different parameters such as temperature, pH, and nitrogen source for the optimum growth. Above result is interpreted in the S/N ratio, larger the ratio better the result, so from the given result it is investigated that optimum value for the growth of Pseudomonas *aeruginosa* is: temperature 30^0c, pH 7 and nitrogen source- peptone.

4.7 GROWTH STUDY

Growth pattern of the *Pseudomonas aeruginosa* was studied. In this experiment optimum condition such as: Temperature- 30^0c, pH- 7, Nitrogen source- Ammonium sulfate and Petroleum sludge as carbon source was taken. At every 8 hour of interval sample was taken centrifuged and supernatant was discarded only pellet was kept for taking biomass reading under UV-VIS spectrophotometer at 600 nm. Growth study was performed for 10 days since after 10 days biomass was remain constant. And the growth curve is shown in the given fig: 22.

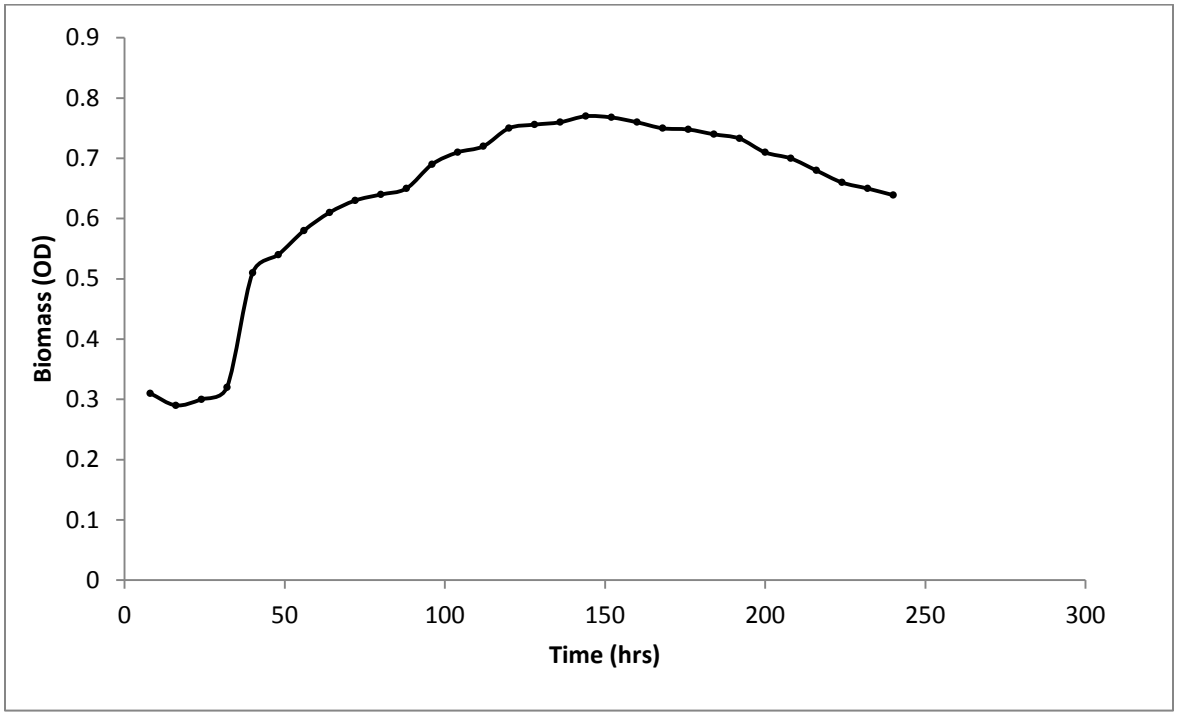

Fig: 22. Growth pattern of Pseudomonas aeruginosa in presence of Petroleum sludge as carbon source.

4.8 DEGRADATION STUDY

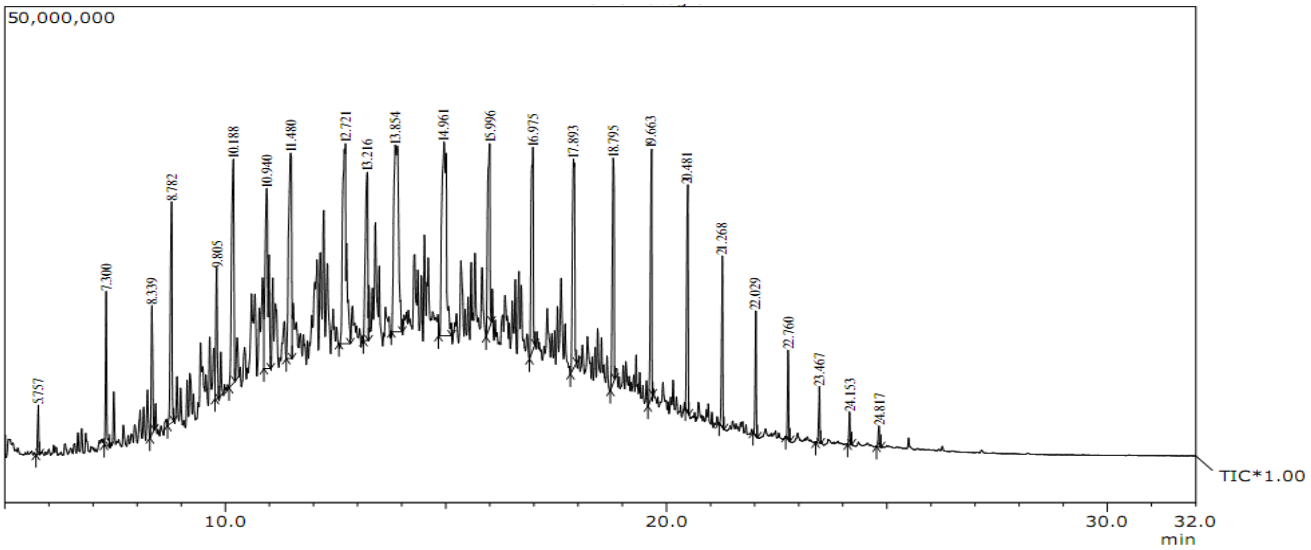

Fig: 23 GC-MS analysis of petroleum sludge(control)

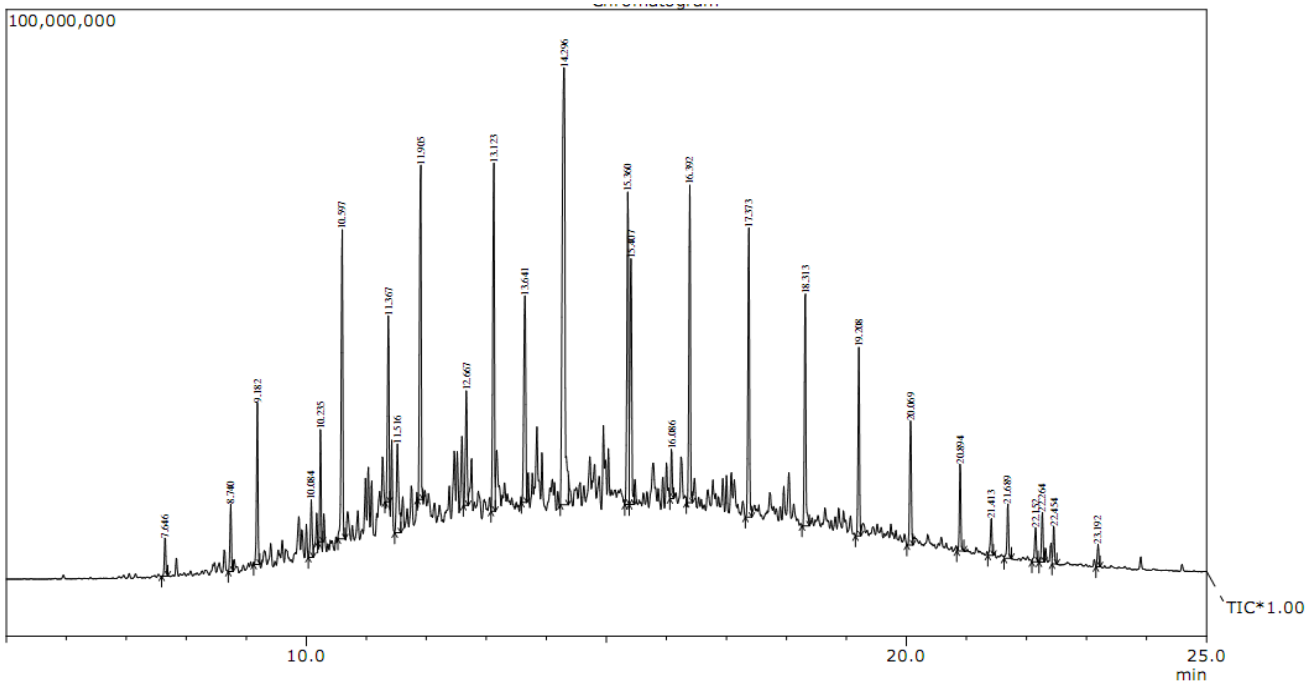

Fig: 24 GC-MS analysis of petroleum sludge after 1st week of degradation.

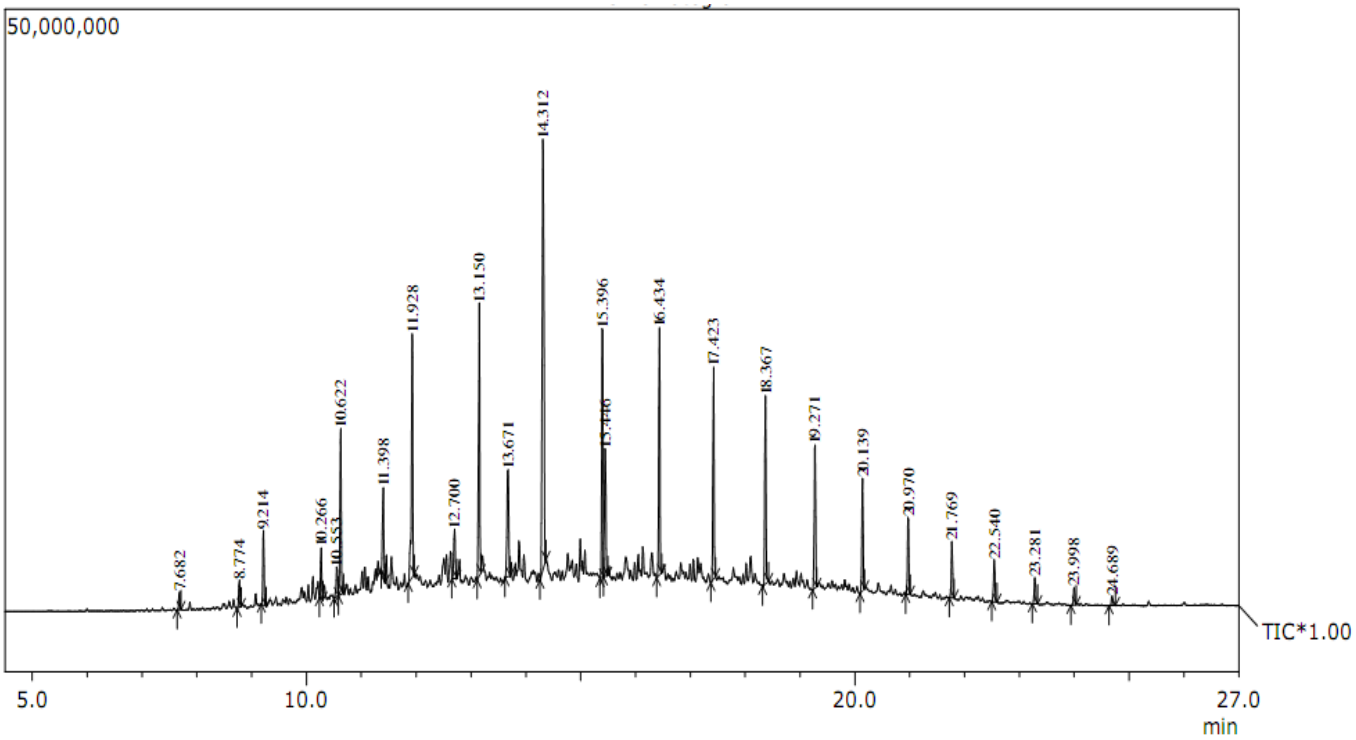

Fig: 25 GC-MS analysis of petroleum sludge after 2nd week of degradation.

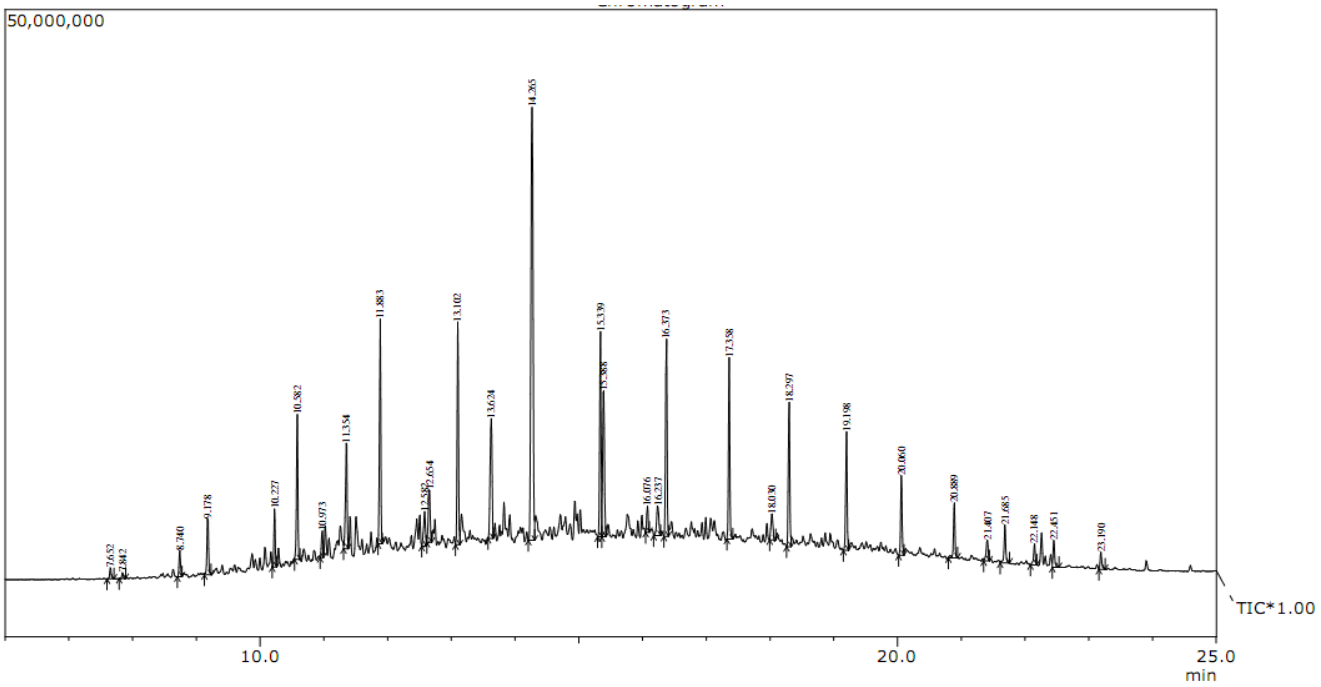

Fig: 26 GC-MS analysis of petroleum sludge after 3rd week of degradation.

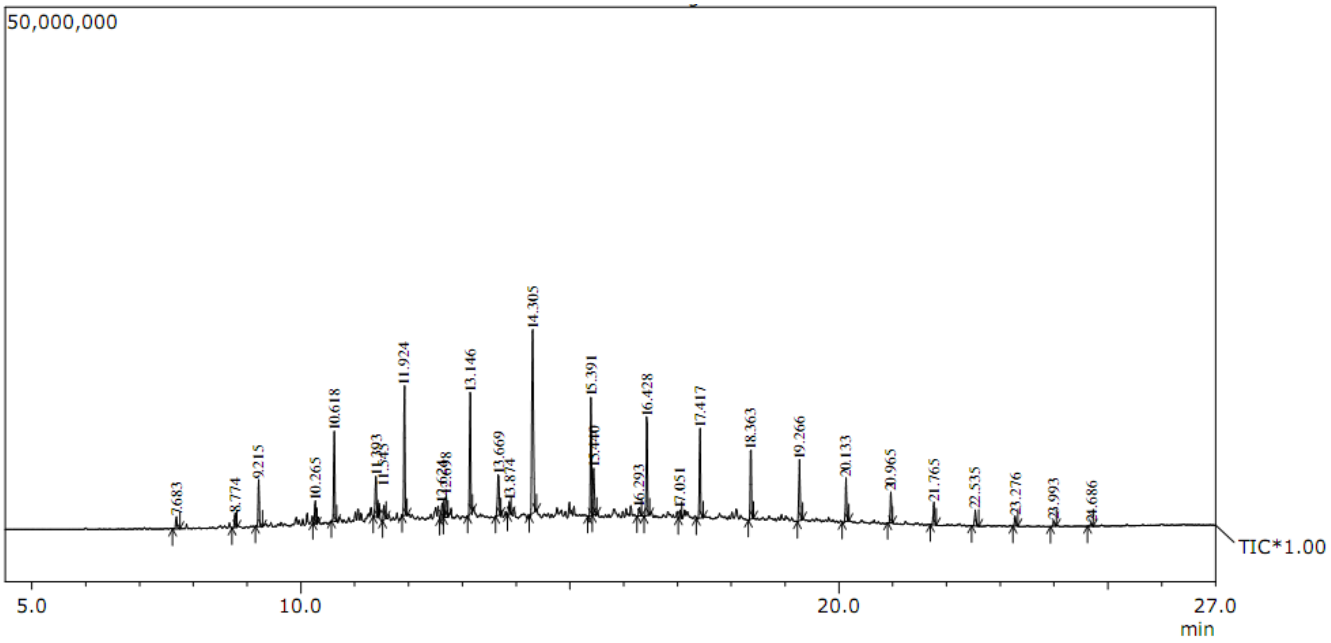

Fig: 27 GC-MS analysis of petroleum sludge after 4th week of degradation.

Table: 13 Degradation pattern of Hydrocarbon of petroleum upto 4 week.

Petroleum Hydrocarbons	Oil sample (Control)	1st week Area%	2nd week Area%	3rd week Area%	4th week Area%
n-Dodecane	2.06	0.83	0.47	0.47	0.47
Dimethyldodecane	1.89	0.93	0.76	0.76	0.70
Tridecane	4.64	3.05	2.13	1.92	1.90
Tetramethylhexadecane	10.35	1.37	1.29	-	-
n-Tetradecane	6.16	5.05	4.98	4.45	-
Tetramethylpentadecane	6.34	3.73	3.73	-	-
n-Pentadecane	6.98	6.96	6.95	6.32	-
n-Hexadecane	8.59	-	-	-	-
n-Octadecane	4.81	4.81	4.7	4.6	4.55
n-Eicosane	10.6	6.23	5.88	5.74	2.67
Nonadecane	5.31	-	-	-	-
Eicosane	5.47	0.77	0.51	-	-
n-Heneicosane	5.34	4.99	4.59	4.0	-
n-docosane	5.01	4.05	3.54	-	-
n-Tetracosane	4.22	2.11	1.90	1.76	1.58
Pentacosane	2.32	-	-	-	-
Hexacosane	1.65	-	-	-	-

Degradation study was done for the 4 weeks and its analysis was done by GC-MS method by taking the sample after every 7 days. Above figures 23, 24, 25, 26, 27 are the resulting graph of GC-MS analysis of the sample such as: control, 1st week, 2nd week, 3rd week and 4th week. And table: 13 is for comparing the degradation of petroleum hydrocarbon after every 7 days. From table:13 it is investigated that some hydrocarbons are totally vanished i.e. Tetramethylhexadecane, n-Tetradecane, Tetramethylepentadecane, n-Hexadecane, Nonadecane, Eicosane, n-Docosane, Pentacosane and Hexacosane, and some of the hydrocarbons are removed upto certain limit after that they remain constant such as n-Dodecane, Dimethyldodecane, Tridecane, n-Octadecane, n-Eicosane, n-Tetracosane etc. Therefore from the table it is concluded that there is significant degradation of petroleum hydrocarbon, so Pseudomonas aeruginosa is very efficient bacteria for the removal of petroleum hydrocarbons.

CHAPTER - 5

CONCLUSION

- Adsorption study on the biological fiber was accomplished and analysis of its surface through SEM, EDX and FTIR reported that fiber surface after treating with petroleum sludge get changed and functional group and element density increases. Therefore it is concluded that biological fiber has the ability to adsorb.
- Biosurfactant screening using CTAB- Methylene blue plates was studied and rhamnolipid production was confirmed.
- Parameter optimization was performed using Taguchi method. Different parameters temperature, pH and nitrogen source was optimized and result shows that temperature 30^0c, pH 7 and nitrogen source peptone is optimum for growth of the *Pseudomonas aeruginosa.*
- Growth rate was also studied during the degradation by taking optimum condition temp 30^0c, pH 7, peptone and petroleum hydrocarbon as nitrogen and carbon source respectively. Growth study showed a maximum biomass growth upto 150 hrs and there after it started decreasing.
- Degradation of Petroleum hydrocarbon was studied for 4 weeks and was analyzed using GC-MS method. It is concluded that degradation of petroleum hydrocarbon through microbial mat is very efficient and feasible. Therefore it can be applied for the bioremediation of oil spill and water bodies.

Suggestions for Future work

- On site application of the microbial mat to the oil contaminated water should be examined.
- Modification of fiber with some chemicals can be studied to evaluate its adsorption capacity.
- A microbial mat can be designed in which both heavy metal degrading microorganism and hydrocarbon degrading microorganism can be immobilized simultaneously.

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